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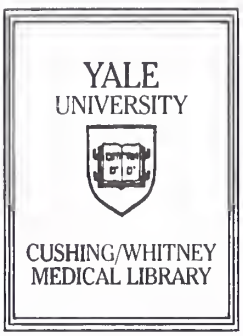
MICROVASCULAR OSCILLATIONS ELICITED LOCALLY BY COMBINED IONTOPHORESIS OF PHENYLEPHRINE AND ACUPRESSURE

Frederick C. Lewis, Jr.

Yale University

2002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Microvascular Oscillations Elicited Locally by Combined Iontophoresis of Phenylephrine and Acupressure

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
Frederick C. Lewis, Jr.
2002

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MICROVASCULAR OSCILLATIONS ELICITED LOCALLY BY COMBINED IONTOPHORESIS OF PHENYLEPHRINE AND ACUPRESSURE. Frederick C. Lewis, Michael Scannell, Julie Park, and David G. Silverman. Department of Anesthesiology, Yale University School of Medicine, New Haven, CT.

This study tested the hypothesis that organized vasomotion of the peripheral microvasculature consistent with cholinergic oscillatory control could be elicited by local iontophoresis of phenylephrine and that the response would be enhanced by acupressure at the Lung point of the ear (a method known to increase vagal tone).

Heart rate, respiration, and forehead flow (via a $1mm^2$ laser Doppler surface probe) were monitored in 8 healthy volunteers. Forehead flow was measured at three sites receiving iontophoresed phenylephrine, iontophoresed normal saline (NS), or no iontophoresis (control site), respectively. Volunteers received iontophoresis for an average of 30 seconds, followed by a ten-minute rest period, then bilateral ear acupressure for two minutes.

The phenylephrine site showed a 25% reduction in flow following iontophoresis ($p<0.01$ using a paired t test), while iontophoresis induced no significant change in flow at the NS or control sites. During the period of ear acupressure, the phenylephrine site showed an increase in the amplitude and organization of oscillatory activity as documented by an increase in intermediate-frequency (IF, 0.12-0.18 Hz) spectral power when compared with the other two forehead sites and with the phenylephrine site at baseline ($p<0.01$ for all comparisons). This increase in forehead oscillatory power, which is consistent with parasympathetic but not sympathetic oscillatory activity, was independent of respiration and of the major oscillatory power of the ECG. No obvious or consistent change occurred in heart rate, standard deviation of heart rate, or heart rate oscillatory power in the IF range, suggesting that there was no systemic effect from either the phenylephrine or the acupressure.

These findings indicate that IF microvascular oscillations can be elicited by local iontophoresis of phenylephrine in combination with enhancement of vagal tone by acupressure. The study provides a model for investigating effects of acupuncture and may help advance the development of clinical monitoring of autonomic function.

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Introduction

The existence of rhythmic variations in biological systems has been recognized for centuries. Oscillations in cardiovascular variables such as heart rate and blood pressure have been appreciated since at least 1733, when Stephen Hales noted beat-to-beat variations in these variables while performing the first quantitative measurements of arterial blood pressure. Since then, many others have described variability in heart rate and blood pressure (1,2). More recently, quantification of these variations has been performed using computer techniques such as power spectral analysis (3,4,5), with the most common of these techniques being Fourier analysis. The fast Fourier transformation (FFT), a computer approximation of the original Fourier Series, allows for the quantification of repeating signals in biological systems over a range of frequencies.

Using the technique of FFT, investigators have identified several discrete frequency bands which account for the majority of variability in heart rate and blood pressure. Low-frequency (LF) fluctuations, below 0.12 Hz, are jointly mediated by the sympathetic and parasympathetic nervous systems (5,6). High-frequency (HF) fluctuations, in the range of 0.12-0.30 Hz, are mediated solely by the parasympathetic nervous system, as evidenced by their elimination with atropine but not by propranolol (6,7). Additionally, the sympathetic nervous system is believed to be incapable of mediating fluctuations at frequencies higher than 0.12 Hz because of the complex second-messenger system involved in adrenergic transmission (8).

A portion of the high frequency component of heart rate and blood pressure variability has been ascribed to vagally mediated changes which occur at the heart in synchrony with respiration. Inspiration produces negative intrathoracic pressure and decreased pulmonary vascular resistance, which in turn lead to increased venous return to the heart and increased stroke volume. The inspiratory decrease in thoracic pressure also leads to an acceleration of heart rate due to a parasympathetic withdrawal triggered by baroreceptor unloading (9). High-frequency

variability in blood pressure, rather than representing fluctuations in vagal efferent activity to the smooth muscles of the vasculature, is thought to result from changes in stroke volume influenced by the status of the respiratory cycle and its effect on venous return (10). Thus cycles of heart rate acceleration and increased stroke volume occur with each inspiratory phase, setting them equal to the respiratory frequency. Therefore, assuming a person is breathing at a rate of at least 12 breaths per minute, the high-frequency range of fluctuations can be further subdivided into intermediate-frequency (IF, 0.12-0.18 Hz) and respiratory frequency (RF, 0.18-0.30 Hz) bands.

In addition to heart rate and blood pressure variability, rhythmic variation has also been demonstrated in the diameters of small arteries, arterioles, and venules. Termed vasomotion, this phenomenon was described by Nicoll and Webb based on their microscopic observation of bat wings (11,12). Fagrell and colleagues later discovered this oscillatory activity in the microvasculature of human skin by using video microscopy, positioning two small videophotometric windows along a monitored capillary (13). In the early 1980s the technique of laser Doppler flowmetry made possible the noninvasive continuous monitoring of microvascular blood flow in skin (14,15,16). Laser Doppler flowmetry monitors flow in the 10-60 capillaries 500-700 nm below the skin surface by detecting frequency shifts of laser light as it is deflected by moving red blood cells in the capillaries under a 1-mm^2 surface probe. The standard laser Doppler output is red blood cell (RBC) flux, or the product of the number of moving RBCs in the microcirculatory vessels under the probe (i.e., the number that cause a Doppler shift) and their velocities (i.e., the magnitude of the shift). However, the vessels in which the flux is measured are predominantly capillaries and postcapillary venules of the upper horizontal plexus which exhibit little change in vessel diameter. Therefore, oscillations in laser Doppler flux are ascribed to propagation of oscillatory activity in the smooth muscle of precapillary sphincters upstream of the capillary bed (17).

Although the physiological role that vasomotion serves is uncertain, one of the most compelling theories is that it may help to direct blood flow to certain body regions. Poiseuille's

law states that the resistance of a vessel is inversely proportional to the fourth power of the vessel's diameter. Wilkin points out that "the resistance of a vessel with constant diameter is notably greater than that of a vessel with the same average diameter in which the diameter changes sinusoidally" (18). Thus oscillations, by creating over time a decreased vascular resistance, may be a physiological mechanism for redirecting blood flow or even for compensating for a state of relative ischemia.

By combining laser Doppler flowmetry with power spectral analysis, researchers have quantified the frequencies of microvascular oscillations (10,19,20,21). Furthermore, investigators have demonstrated that, similar to heart rate and blood pressure periodicity, different frequency ranges of microvascular oscillations appear to correlate with varying degrees of sympathetic and parasympathetic mediation.

The origin of microvascular oscillations is unknown and may be multifactorial. Nicoll and Webb described vasomotion as the inherent property of vascular smooth muscle cells to exhibit spontaneous contractions (11). Several researchers believe that this steady state of "background" oscillatory activity can be altered by local metabolic demands (22,23,24). Siegel proposed a biochemical explanation for the rhythmicity of vascular smooth muscle based on the kinetics of the allosteric enzyme phosphofructokinase (25). Applying known enzyme kinetics and tissue concentrations, he described a pathway through which changing substrate concentrations could cause varying activity of the enzyme, leading to oscillations in glycolysis and ultimately to oscillations in membrane potential and cell contractility. Wilkin demonstrated that microvascular oscillations at 0.1 Hz are particularly prominent during the period of recovery from postocclusive reactive hyperemia and aldehyde-induced hyperemia (18). Citing the biochemical pathway described by Siegel as the source of the oscillations, Wilkin proposed that the stimulus for oscillations in both cases is the physical or pharmacologic stretch of myogenic cells. Following this stimulus, the oscillations become most prominent during the return from

vasodilation (accompanied by hyperpolarization of smooth muscle cells) to resting sympathetic vasoconstrictor tone.

Other theories on the source of microvascular oscillations account for autonomic regulation of the microvasculature. LF oscillations (<0.12 Hz) are considered to be a marker of sympathetic activity in heart rate and blood pressure. At the microvascular level, Bernardi and colleagues demonstrated that skin blood flow oscillations at 0.1 Hz could be enhanced by maneuvers (such as tilting) which increase sympathetic tone and blunted by pharmacologic sympathectomy (20). As with heart rate and blood pressure, HF microvascular oscillations (>0.12 Hz) are thought to represent purely parasympathetic influence because the sympathetic nervous system's second-messenger system makes it incapable of transmitting signals at these higher frequencies (8). The respiratory frequency is known to account for a portion of the HF range microvascular oscillations via mechanical transmission of variations in cardiac stroke volume, in the same manner as discussed above for the respiratory influence on blood pressure variability.

Using the squared coherence function, Bernardi *et al.* measured the correlation between oscillations in skin blood flow of the finger and oscillations in blood pressure and heart rate (10). The squared coherence function, a type of bivariate analysis, allows determination of whether two signals oscillating at the same frequency maintain a stable phase relationship. They discovered that HF oscillations in blood pressure and heart rate precede those in the skin blood flow, indicating that these oscillations in the microvasculature reflect downstream transmission of vagally mediated fluctuations in cardiac tone. By contrast, LF oscillations were found to originate in the skin, as they preceded these same oscillations in blood pressure and heart rate.

Several additional observations are consistent with the theory of autonomic influence of microvascular oscillations. Mevio and Bernardi compared skin blood flow oscillations in the forearm and nasal mucosa using laser Doppler flowmetry (26). The nasal mucosa is known to have rich autonomic innervation, both sympathetic and parasympathetic, whereas the innervation

of forearm skin vessels, while mixed, is more sympathetic than parasympathetic. The investigators found that increasing the respiratory frequency—and thereby the overall frequency of any parasympathetic component—caused an increase in oscillatory frequency of blood flow in the nasal mucosa but not in the forearm skin. They also discovered that the tilt test maneuver (which is known to be a sympathetic stimulus) caused a significantly larger decrease in blood flow oscillatory frequency in the nasal mucosa than in the forearm skin, suggesting that the parasympathetic relative to sympathetic innervation is greater in the nasal mucosa vasculature than in that of forearm skin. Furthermore, when Salerud and colleagues measured oscillatory frequencies at multiple sites in one subject, they found frequencies in the forehead, upper arm, forearm, and foot of 10.0, 7.2, 6.3, and 5.6 cycles per minute, respectively. The finding of higher oscillatory frequencies proximally is consistent with the known higher ratio of parasympathetic to sympathetic innervation at more central regions of the body.

In a further study of cutaneous laser Doppler flowmetry, Silverman and colleagues measured the RBC flux at the volar forearm and the palmar surface of the finger at baseline and after systemic infusion of phenylephrine (27). As expected, the laser Doppler flux decreased at the finger site following phenylephrine administration. However, flux values in the forearm increased during the infusion, suggesting a potential homeostatic response to increased plasma concentrations of an α_1-receptor agonist. While the vessels of both the finger and forearm are known to be richly innervated with adrenoceptors, the forearm skin vessels are also under cholinergic regulation (28,29,30,31). Additionally, the increase in flux seen in the forearm was consistent with vagally mediated baroreceptor responses noted in other settings, such as carotid sinus stretch and body tilting (32,33). Furthermore, the homeostatic nature of the vagal response to infused phenylephrine was demonstrated by the finding that the pressure increase induced by phenylephrine was markedly increased after vagal block with atropine (34). Thus it was reasonable to hypothesize that the disparity between finger and forearm reaction to an α_1-agonist

was due to a cholinergically mediated homeostatic response in the microvasculature of the forearm.

Based on the results of this study and the knowledge that cholinergic activity is primarily responsible for oscillations in heart rate (heart rate variability), Silverman and colleagues next hypothesized that cholinergic activity might induce oscillations in the microvasculature which might be activated in the presence of a vasoconstrictive stimulus (35). To investigate this hypothesis, they used laser Doppler flowmetry to measure flux in the forehead and the palmar surface of the finger at baseline and after intravenous infusion of phenylephrine. In the forehead, the average flux remained constant during the infusion and developed prominent oscillations in the IF range (0.12-0.18 Hz). The cholinergic nature of these oscillations was confirmed by their ablation upon intravenous administration of atropine. LF oscillatory power did not change during any phase of the study. By contrast, finger flux decreased by 50% during phenylephrine infusion and did not exhibit IF oscillations. The investigators concluded that the IF oscillations of the forehead microvasculature were consistent with cholinergic oscillatory control.

Although these findings suggest a cholinergic parasympathetic control of the vasculature, traditionally autonomic regulation of the peripheral vasculature is believed to be almost entirely sympathetic. Aside from its role in "local functional hyperemia" (36) such as the erectile system of the penis, acetylcholine (ACh) has been considered an "outcast as a vasomotor transmitter" (36), and the parasympathetic nervous system is viewed as having "virtually no effect on peripheral resistance" (37). This belief persists despite the identification of ACh receptors in vessel walls (36,38), ACh-induced vasodilation upon iontophoretic delivery or continuous infusion (39,40), and evidence of a parasympathetic vasodilator mechanism in the lower gingiva of cats (41). Nevertheless, the traditional consensus as to the lack of physiologically relevant cholinergically mediated vasomotion suggests that these IF oscillations do not originate in the microvasculature, but rather represent the downstream transmission of oscillations in cardiac stroke volume in response to a vagomimetic challenge and thus would be transmitted via pressure

and flow waves to the periphery in a manner similar to the transmission of respiratory-induced fluctuations in heart rate and blood pressure. Therefore, in order to suggest that these oscillations represent a local parasympathetic control of the microvasculature, it is necessary to document a lack of association between cholinergic oscillatory control at the heart and at the microvasculature.

To demonstrate this lack of association, Silverman and colleagues measured laser Doppler flux in the forehead and ventral forefinger of eight subjects first at baseline and then during the infusion of phenylephrine to achieve a 50% decline in finger flux (42). Throughout the study subjects breathed in time to a metronome at 12 breaths/minute (0.20 Hz) to fix the frequency of respiratory oscillations. Although there was the anticipated baroreceptor-mediated decline (10-15%) in heart rate with phenylephrine infusion, there was no significant change in the magnitude of heart rate oscillations, which persisted at the respiratory frequency during both the baseline and infusion phases. In contrast, the forehead flux developed increased oscillations during the infusion phase, with a unique peak at approximately 0.14 Hz. Both the heart rate and the forehead flux oscillations were eliminated by the infusion of atropine, confirming their cholinergic nature. Additionally, continuous monitoring of blood pressure via a finger plethysmographic technique in several subjects confirmed that the oscillations in forehead flux occurred at a frequency distinct from that of systemic blood pressure. Furthermore, the simultaneous use of multiple forehead laser Doppler probes revealed that the oscillations at different forehead sites were not necessarily in phase with one another, indicating that the oscillatory control was at the level of the microvasculature rather than at a central location which would have coordinated the oscillations at the different sites. The peripheral mediation of the phenylephrine-induced oscillations was further confirmed by their elimination by topical application of a eutectic mixture of local anesthetic.

These observed cholinergically mediated oscillations during infusion of an α_1-agonist are consistent with reports of ACh-induced release of nitric oxide in response to changes in

intraluminal pressure (43,44). Indeed, nitric oxide has been observed to "limit the work of perfusion…by coordinating the behavior of resistance vessels" (45). In addition, the 5-10 second half-life of nitric oxide is consistent with the period of the HF oscillations. A homeostatic process such as the described cholinergic oscillatory control would help explain the observations that subcutaneous injection of phenylephrine does not cause local blanching (46) and that addition of vasoconstrictive drugs to an *in vitro* vessel preparation induces an endothelium-dependent compensatory vasodilatory response (47). Although there appears to be local control of this oscillatory response based on the above study by Silverman and colleagues, it is unclear whether the entire pathway mediating the oscillations is confined to the microvasculature or whether there is a hierarchical control system similar to that of the smooth muscle of the gastrointestinal tract, which is under both local and vagal control.

A microvascular homeostatic oscillatory mechanism may have special significance in certain cardiovascular disease states. The addition of ACh to *in vitro* vessel preparations causes vasodilation even in the context of vasoconstrictive drugs, but this ability to dilate is lost in the presence of endothelial injury (47,48). Diabetic autonomic neuropathy and hypertension are both associated with loss of autonomic activity and development of endothelial injury, and several studies have examined changes in oscillatory behavior at both the central (i.e., heart rate and blood pressure) and microvascular level in patients with these diseases.

In a study of hypertensive patients being tilted from supine to upright, the LF component of heart rate fluctuations increased and the HF component decreased much less in hypertensive subjects than in the control group, and the LF components of skin blood flow increased only in control subjects (49). These findings suggest sympathetic and parasympathetic impairment in hypertensive subjects.

Multiple studies have demonstrated impairment of sympathetic-range vasomotion in diabetics. Stansberry and colleagues found the amplitude of very low-frequency vasomotion (1-2 cycles per minute) to be much lower in diabetic patients than in control subjects and that this

decreased vasomotor amplitude also correlated with loss of function of thinly myelinated C-fibers (50). Bernardi and colleagues found the power of 0.1 Hz microcirculatory fluctuations to be significantly lower in diabetic than in control subjects (51). Aso and colleagues discovered that the vasoconstrictor response (measured in the big toe) to deep inspiration (which is known to elicit vasoconstrictive effects) was significantly decreased in diabetic patients compared with healthy subjects (52). Furthermore, in diabetic patients the vasoconstrictor response was negatively correlated with the duration of diabetes, the median motor and sensory nerve conduction velocities, resting heart rate variability, and postural fall in systolic blood pressure.

Other studies have examined dysfunction in cholinergic and parasympathetic activity in diabetics. Bernardi and colleagues found an absence of power in the HF band of the heart rate variability power spectrum in diabetic patients (53). In addition, loss of HF oscillatory activity in heart rate and blood pressure in diabetics was found to be associated with poor wound healing (54). Morris and colleagues found the vasodilatory effects of both acetylcholine and sodium nitroprusside (a nitric oxide donor) to be attenuated in diabetic patients compared with healthy controls, suggesting that endothelial and/or smooth muscle function may be impaired in the skin microcirculation of diabetic patients (55). Given the evidence that diabetic patients have both impaired centrally mediated parasympathetic oscillations and attenuated cholinergically mediated vasodilation, it is reasonable to suspect that they may also have a dysfunction of parasympathetic frequency-range oscillations at the microvascular level.

If microvascular cholinergic oscillatory control serves a homeostatic function that is impaired in patients with cardiovascular disease, it would be useful to have a method of testing this function in patients at risk for developing these diseases before they show clinical signs of impairment. In fact, there is mounting evidence that such autonomic impairment precedes clinical signs. The diabetic patients found in Bernardi's study to have decreased sympathetic-range microcirculatory fluctuations had no clinical symptoms of autonomic dysfunction (51); Aso and colleagues' study showing that diabetics had decreased vasomotor reflexes in response to

deep inspiration was performed on diabetic patients without peripheral vascular disease or evidence of autonomic dysfunction (52). These findings, coupled with evidence that sympathetic nerve failure in diabetics occurs earlier than previously thought (56), suggest not only that microvascular oscillatory dysfunction may contribute to the pathogenesis of these diseases, but also that its early detection may help in the diagnosis and treatment of patients at risk for cardiovascular disease.

As mentioned previously, it is unclear whether the entire pathway mediating IF microvascular oscillations is confined to the vasculature or whether there may be a hierarchical control involving more proximal regulation. To address this question, a method of eliciting the IF microvascular oscillations previously seen on phenylephrine infusion was sought that could be both performed and measured at a local level. Such a method would ideally be as noninvasive as possible to avoid activating local pain pathways which could affect blood flow and interfere with or prevent the oscillations. A review of the literature suggested that the least invasive means of administering phenylephrine locally is via iontophoresis.

Iontophoresis is a technique which uses small, physiologically safe amounts of electric current to administer soluble chemicals and drugs to a small surface region of the body such as the finger, forearm, or forehead. By using an electrode of the same polarity as the charge of the drug, the drug is driven into the skin by electrostatic repulsion in a quantity proportional to the current applied. This technique avoids the pain and tissue disruption associated with needle injection and the potential effects of systemic drug administration. Iontophoresis has found widespread clinical applications in dermatology (57,58) and is also used in physical therapy, primarily for the delivery of lidocaine and dexamethasone (60). The Department of Pediatrics at our own institution regularly uses a commercially available iontophoresis system to deliver a solution of lidocaine HCl 2% + epinephrine 1:100,000 prior to placement of intravenous catheters (Iomed, Inc., Salt Lake City, Utah).

Several investigators have already shown that a wide variety of drugs, including norepinephrine, phenylephrine, acetylcholine, atropine, lidocaine, and nitroprusside have been administered safely and effectively by iontophoresis (28,60,61,62,63,64). Indeed, phenylephrine and norepinephrine administered iontophoretically induce local vasoconstriction (63), while acetylcholine and nitroprusside cause the anticipated vasodilatation (62). However, these studies measured changes in overall flow which were produced by iontophoresis of drugs, rather than measuring changes in microvascular oscillatory patterns.

While awaiting the delivery of a suitable iontophoresis system, our research team tested subcutaneous injection of phenylephrine as a potential local delivery method. Using a 24-gauge needle, 0.1 ml of phenylephrine 1:25,000 was injected subcutaneously at a site on the forehead, and 0.1 ml of normal saline was injected subcutaneously at a different forehead site. Preliminary results indicated the occurrence of vasoconstriction and the development of IF oscillatory activity at the phenylephrine site almost immediately after injection. However, the injection was found to produce a wheal at the skin surface which may have caused local compression of blood flow and may have altered the distance from the laser Doppler probe to the capillary bed in which flow was being measured. Therefore, comparison of laser Doppler readings before and after injection may have been unreliable. Additionally, the injection of drug was found by multiple subjects to be painful. Thus there was the possibility of activation of a local pain reflex, with release of histamine and other vasoactive elements which may have affected local blood flow.

If IF microvascular oscillations represent a homeostatic parasympathetically mediated response, it is reasonable to hypothesize that maneuvers which increase vagal tone may increase the power of these oscillations. One method which has been postulated to affect the cardiovascular system via the autonomic nervous system is acupuncture. Investigators have shown that acupuncture at different skin locations appears to activate sympathetic or parasympathetic responses (or both) to different degrees (65). For example, investigators examining the effects of manual acupuncture on LF and HF heart rate fluctuations found that

acupuncture at the LI-4 (Hoku) point of the hand elicited both sympathetic and parasympathetic responses, while comparable stimulation of the Lung point of the ear (inferior hemi-conchae) caused a purely parasympathetic response (65). Therefore, use of an acupuncture technique to stimulate the Lung point in a subject may have the potential to enhance the power of any parasympathetically-mediated microvascular oscillatory activity that may be occurring.

Purpose and Hypothesis

We believe that there exists a novel homeostatic mechanism in the cutaneous microcirculation which is activated in response to a vasoconstrictive stimulus. To provide further evidence of this mechanism, and to help characterize it with respect to the possible location or locations of the stimulus for its activation, we hope to elicit high-frequency cutaneous microvascular oscillatory activity in the forehead via the noninvasive local administration of phenylephrine by iontophoresis. In so doing, we hope to replicate the effects seen in the forehead following phenylephrine infusion by stimulating the response at a local level. Research has already demonstrated that monitoring microvascular oscillatory activity provides information about the presence and severity of sympathetic dysfunction, even before clinical signs develop, particularly in the setting of diabetes (49,50,51). Having the ability also to monitor a parasympathetically-mediated response would expand the value of monitoring cutaneous flow periodicity by providing information on the status of both the sympathetic and parasympathetic branches of the autonomic nervous system.

We hypothesize that the iontophoretic delivery of phenylephrine to the forehead skin will induce changes in forehead microcirculatory flow similar to those induced by the systemic administration of phenylephrine by Silverman and colleagues. Specifically, we anticipate that our monitoring of laser Doppler flow in the forehead will reveal changes in spectral components of variability indicative of intermediate-frequency (0.12-0.18 Hz) activation and that these changes will be specific to the site at which phenylephrine is administered. We further hypothesize that these changes will be enhanced by acupressure (a form of acupuncture) at the Lung point of the ear.

Methods

The local institutional review board approved the protocol for the present study; all subjects gave their informed, written consent.

The study participants, all healthy nonsmoking males, were instructed to refrain from caffeine and other vasoactive substances for several hours prior to each study session. At the study's start, the subject lay recumbent with arms, legs, and head maintained at heart level. The room temperature was regulated at 71 ± 1°F. Surface electrodes were applied for monitoring ECG and respiration. Two iontophoretic drug delivery pads (Iomed, Inc.) were prepared by filling one with 1 ml of 1,600 mcg/ml phenylephrine and the other with 1 ml of 0.9% normal saline (NS) diluted 1:6 in sterile water. One laser Doppler flowmetry probe (Periflux 2B, Perimed, Sweden) was inserted through each of the two iontophoretic pads, which were then affixed to different sites on the subject's forehead so that the tips of the laser Doppler probes rested against the skin, flush with the surfaces of the pads. A third laser Doppler probe was taped to a third site on the forehead away from both iontophoresis pads. Thus laser Doppler probes were affixed to three forehead sites: a phenylephrine delivery site, a normal saline delivery site, and a control site (without an iontophoretic drug delivery pad). A current delivery device was attached to each of the two iontophoretic pads, with the positive lead attaching to the drug delivery pad and the negative lead connecting to a dispersive pad which was affixed to the subject's upper back.

During all study phases, the ECG, respiration, and laser Doppler flux were recorded at 250 Hz with a microprocessor-based system consisting of analog-to-digital converters, commercially available data acquisition software (Snapmaster, HEM Data Corp), customized software for beat detection and annotation, and commercially available systems for Fourier analysis (Snapmaster, HEM Data Corp; Excel, Microsoft).

Following the application of our monitoring devices, subjects were allowed to rest comfortably for 5-10 minutes before being asked to breathe in synchrony with an audible metronome at a rate of 12 breaths per minute (0.2 Hz). Metronome breathing was continued throughout the remainder of the study except where otherwise noted. Baseline data were collected for a period of 2 minutes. Both iontophoresis current delivery devices were then turned on for a period of 10 to 30 seconds at a current of 2 mAmp. Following the period of iontophoresis, the subject rested while the investigators monitored the laser Doppler flow patterns for the appearance of oscillatory activity and/or a decrease in flow at the phenylephrine site indicative of vasoconstriction. If no change in flow was detected at either site after 5 additional minutes of recording, iontophoresis was repeated at both sites for an additional 10 to 30 seconds, after which flow was again monitored for 5 minutes. This sequence was repeated as necessary until a maximum total duration of iontophoresis of 2 minutes was reached.

When a >10% decrease in flow at the phenylephrine forehead site became evident, the subject was allowed to rest for approximately 10 minutes. After this rest period, an investigator applied manual fingertip acupressure to the Lung point (inferior hemi-conchae) of both of the subject's ears for a period of 2 minutes, after which the subject was allowed to rest for an additional 2 minutes.

Analysis

The frequency and power of the laser Doppler flux signals were characterized by auto-power spectral density (APSD) frequency-domain analysis. The computer resampled the laser Doppler flux signals at 5 Hz, a rate, according to Nyquist's Theorem (66), more than sufficient to enable identification of oscillatory frequencies within our range of interest (0.05-0.30 Hz). We then selected multiple segments ranging from 2 to 3 minutes from each of the three laser Doppler site tracings and from these generated sets of $APSD_{phenyl}$, $APSD_{NS}$, and $APSD_{control}$ in each subject

using a traditional Parzan window, $G_{aa} = [ave(S_aS^*_a)]/df$, wherein G_{aa} is instantaneous amplitude spectral density of channel a, S_a is instantaneous amplitude spectrum of channel a, S^*_a is complex conjugate of S_a, and df is frequency resolution (0.005 Hz). The laser Doppler flux time segments selected for frequency domain analysis at each site were as follows: 1) baseline; 2) during maximal vasoconstriction at the phenylephrine site; 3) during the period of maximum IF range oscillatory power for each site (after iontophoresis but before ear acupressure); 4) during ear acupressure; 5) immediately after ear acupressure. Thus the analyzed time segments represent simultaneous recordings at all three laser Doppler sites with the exception of the "maximum IF range oscillatory power" phase, which was selected as the period of maximum IF oscillations at *each* site.

After the flux tracings from each forehead site and time segment had been characterized by Fourier analysis, for each APSD the 0.01-Hz wide bin with maximum power ("maxbin") in the IF range was identified. The IF range maxbin power at all three sites was compared, for all studies, using one-way analysis of variance (ANOVA). A paired t-test was then used to compare, for all studies, the IF range maxbin power at the phenylephrine site versus that at the normal saline site and at the phenylephrine site versus the control site.

In addition to comparing the maxbin power at the phenylephrine site with that of the other sites, we compared the maxbin power during each time segment with the power at baseline to quantify the overall change in maxbin power. Dividing the maxbin power at each segment by the baseline power proved to be an unreliable technique because the baseline powers, while generally negligible, varied enough (0.01-0.07 at the phenylephrine site) to cause excessive distortion of such ratios. We therefore decided to quantify changes in maxbin power in the following two ways. First, for each forehead site we calculated the maxbin power at each phase minus the power at baseline. Additionally, we used a paired t-test to compare, for each forehead site, the maxbin power during each time segment with the power at baseline.

One study was selected for analysis of the beat-to-beat percent change in flux. Using software for detection of R waves on the ECG tracing, the laser Doppler flux during each heartbeat was recorded. The percent change in flux from one beat to the next was calculated using the formula $[((n_2-n_1)/n_1)*100]$ where n_1 represents the laser Doppler flux at one heartbeat and n_2 represents the flux at the subsequent beat.

In four of the studies, the time-domain indices of the heart rate and R-R interval (the time in milliseconds between two consecutive R-waves as measured by an ECG) were reported as mean ± standard deviation. For each time segment, the ECG tracing itself was also sampled at 250 Hz, resampled at 5 Hz, then characterized by APSD analysis. The IF range maxbin power of the ECG tracing was recorded for each time segment.

Results

The forehead site which received iontophoresed phenylephrine showed an average 25% decrease in flux following iontophoresis. By contrast, after iontophoresis the NS site showed either no change in flux or a mild increase, while flux at the control site did not change markedly. The phenylephrine site showed an increase in IF range maxbin oscillatory power at approximately 0.125 Hz during the phase of ear acupressure, which was significant compared with the other forehead sites and with the phenylephrine site during the baseline period. No consistent change was seen throughout the study in R-R interval, R-R variability, or IF maxbin power of the heart rate.

Figure 1 shows a representative example of raw data collected during a study. The top portion of the figure portrays the two-minute baseline period, while the bottom portion displays the two-minute period during ear acupressure. Shown for each segment are the ECG tracing, laser Doppler tracings for the forehead phenylephrine and control sites, and the respiratory monitor tracing. The small rises and falls in flux represent downstream transmission of each cardiac systolic contraction. In addition, periodic oscillations in flux can be seen most prominently at the phenylephrine site during ear acupressure, where they show a frequency of approximately seven per minute.

Figure 2 shows the mean laser Doppler flux measured at each of the three forehead sites for each study during the five time periods of interest. Although the "maximum vasoconstriction" phase, shown for all three sites, is based on the period of lowest flux at the phenylephrine site alone, there were no obvious periods of increase or decrease in flux at the other two forehead sites that are not reflected by the values plotted in Figure 2. Table 1 shows the mean flux over all studies at each site during each time period. Also given in this table are p values for a two-tail paired t-test comparing, for each site, the mean flux during each phase with the mean flux at baseline. Because of the skewness of the data, the flux values were also compared by a two-tail paired t-test after *ln* (natural log) transformation, which helps correct for

wide variability of a few single data points. These *p* values are also shown in Table 1. In addition, because multiple t-tests were performed on the same set of data, Bonferroni's method was used to adjust the level of significance in order to reduce the probability of type I error (67). *p* values indicating significance after Bonferroni's adjustment are indicated in the table with an asterisk.

As expected, there is a significant decrease in flux (approximately 25%) at the phenylephrine site following iontophoresis (which occurred between the phases of baseline and maximum vasoconstriction), indicating local vasoconstriction ($p = 0.001$-0.007). The significance of this decrease in flux at the phenylephrine site is maintained after *ln* transformation. By contrast, no significant change in flux is seen at the control site, consistent with there being no systemic effect of the phenylephrine. The variable increase in flux at the normal saline site in several subjects indicates a potential hyperemic effect from iontophoresis itself; this resulted in an overall increase in flux at the NS site during ear acupressure. Although *p* values for the uncorrected data indicate a significant change in flux at the NS site during and after ear acupressure when compared with baseline, no subject developed vasoconstriction at the NS site immediately following iontophoresis.

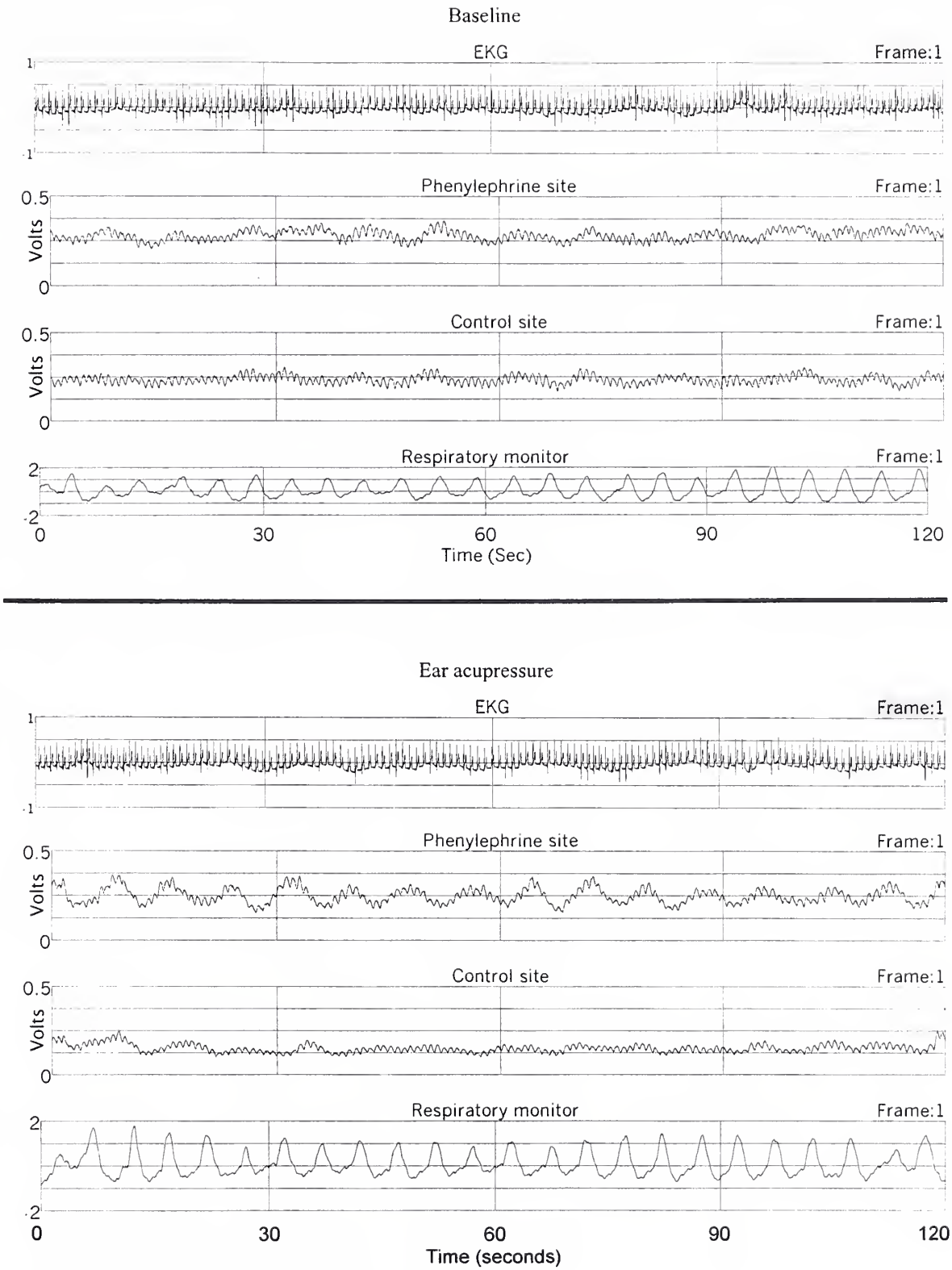


Fig. 1: Raw data collected during a study. The top portion portrays the two-minute baseline period; the bottom portion portrays the two-minute period during ear acupressure.

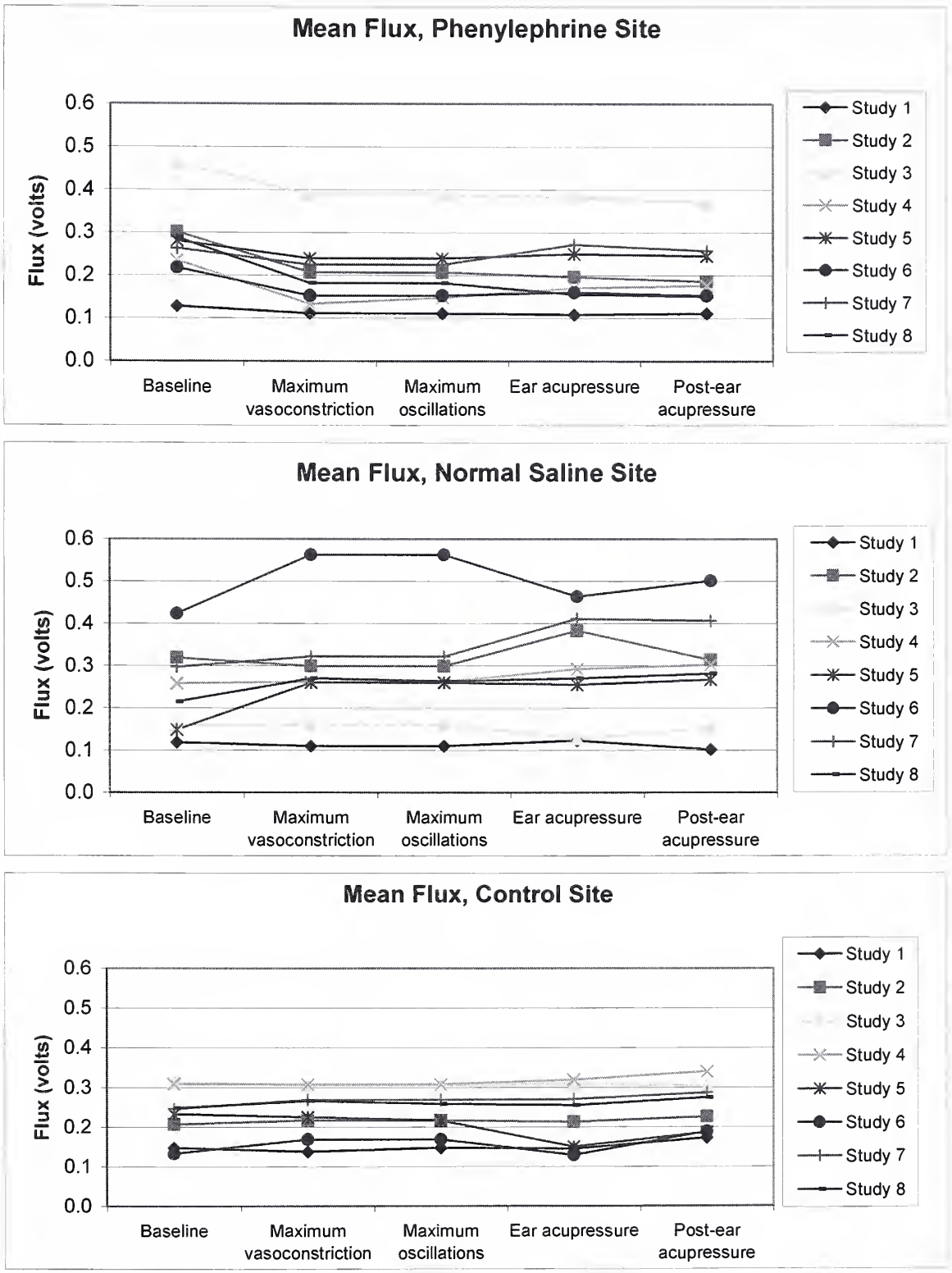


Fig. 2: Mean laser Doppler flux for all studies.

Table 1: Mean (standard error of mean) flux for all studies

	Baseline	Maximum vasoconstriction	Maximum oscillations	Ear acupressure	Post-acupressure
Phenylephrine	0.273 (0.033)	0.204 (0.031)	0.207 (0.030)	0.212 (0.031)	0.206 (0.029)
p		0.001*	0.001*	0.007*	0.005*
p (ln trans)		0.001*	0.001*	0.007*	0.005*
Normal saline	0.243 (0.036)	0.281 (0.047)	0.280 (0.047)	0.291 (0.044)	0.243 (0.036)
p		0.11	0.11	0.026	0.038
p (ln trans)		0.13	0.14	0.07	0.10
Control site	0.230 (0.023)	0.237 (0.022)	0.237 (0.021)	0.224 (0.027)	0.248 (0.022)
p		0.24	0.27	0.64	0.15
p (ln trans)		0.29	0.27	0.52	0.17

Data values indicate the mean flux for all eight studies at the given forehead site and time period. *p* values are for two-tail paired t-test comparing the flux during each time period versus the flux at baseline. The first *p* value in each set represents comparison of the actual flux values between the given phase and baseline; the second *p* value represents comparison after *ln* transformation of the flux values for the given phase and baseline in order to minimize the influence of skewness. *p* values retaining significance after Bonferroni's adjustment are indicated with an asterisk.

Figure 3 shows $APSD_{phenyl}$, $APSD_{NS}$, and $APSD_{control}$ during the baseline time period of the study for which the raw data is shown in Figure 2. Also shown is the beat-to-beat percent change in flux at all three laser Doppler sites during the baseline time period. Figures 4, 5, and 6 illustrate this same set of data during the following time periods: maximum vasoconstriction at the phenylephrine site, ear acupressure, and immediately following ear acupressure, respectively. As can be seen by comparing the figures, the APSD of the phenylephrine site develops a prominent peak at 0.125 Hz during the phases of ear acupressure and post-acupressure, while the other two sites do not develop as much oscillatory power in the IF range (0.12-0.18 Hz). Examining the tracings of beat-to-beat percent change reveals an increased organization of the beat-to-beat changes in flux at the phenylephrine site during and after acupressure compared with the other forehead sites and with the phenylephrine site before acupressure.

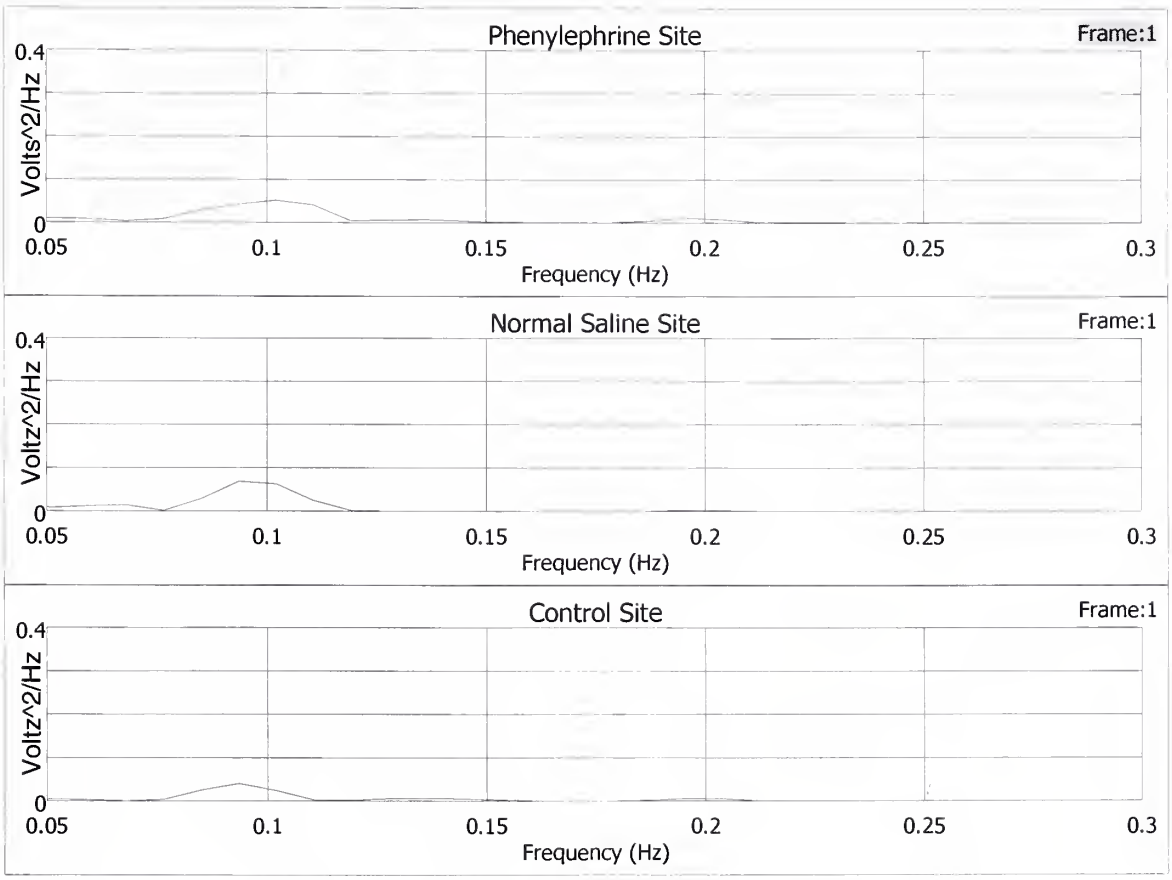
Figure 7 plots the values of IF range maxbin power for each study. Reviewing the graphs, a marked increase in IF maxbin power is noted in almost all subjects during the post-iontophoresis period, ear acupressure, or both. By contrast, with the exception of one subject at the NS site, there is no obvious change in the IF maxbin power at either the NS or control site. For each of the five study phases, the IF maxbin power was compared between all three sites using ANOVA. Because of the appearance of several potentially outlying data points, the data was corrected for skewness by taking the natural log (*ln*) of each maxbin power. By ANOVA, a significant difference exists between the maxbin powers at the three sites during the ear acupressure and post-acupressure phases ($p = 0.0009$ and $p = 0.036$, respectively, for *ln* transformation corrected data).

Table 2 presents, for each forehead site and time segment, the mean maxbin power over all studies. Included in this table are *p* values for a two-tail paired t-test comparing, for all studies, the maxbin power at the phenylephrine site versus the NS site and at the phenylephrine site versus the control site. *p* values for a paired t-test comparing the *ln* transformation of the maxbin power at the phenylephrine site versus the NS site and at the phenylephrine site versus the

control site are also presented in the table. The p values in Table 2 indicate the development of a significantly greater IF maxbin power at the phenylephrine site compared with the other two sites during the phases of ear acupressure and post-acupressure ($p < 0.05$ versus both NS and control sites during both of these time periods). A t-test comparing the *ln* transformation of the maxbin powers during the acupressure phase shows even greater significance than when comparing the actual maxbin powers. Therefore, correcting for skewness in the data by using *ln* transformation increases the significance of the acupressure-phase IF oscillatory power at the phenylephrine site compared with the NS and control sites.

Figure 8 plots, for each study, the maxbin power during each phase minus the maxbin power during the baseline phase (phase-minus-baseline). Table 3 presents, for each forehead site and time segment, the mean phase-minus-baseline value over all studies. Included in this table are p values for a two-tail paired t-test comparing, for all studies, phase-minus-baseline at the phenylephrine site versus the NS site and at the phenylephrine site versus the control site. These p values indicate a significantly greater phase-minus-baseline power at the phenylephrine site versus both the NS and control sites during the period of ear acupressure. Because several of the phase-minus-baseline power values are negative (indicating a higher oscillatory power at baseline than during a later phase), *ln* transformation is not possible for this set of data.

Table 4 displays p values for a paired t-test comparing, over all studies, the IF maxbin power during each phase with the IF maxbin power at baseline.



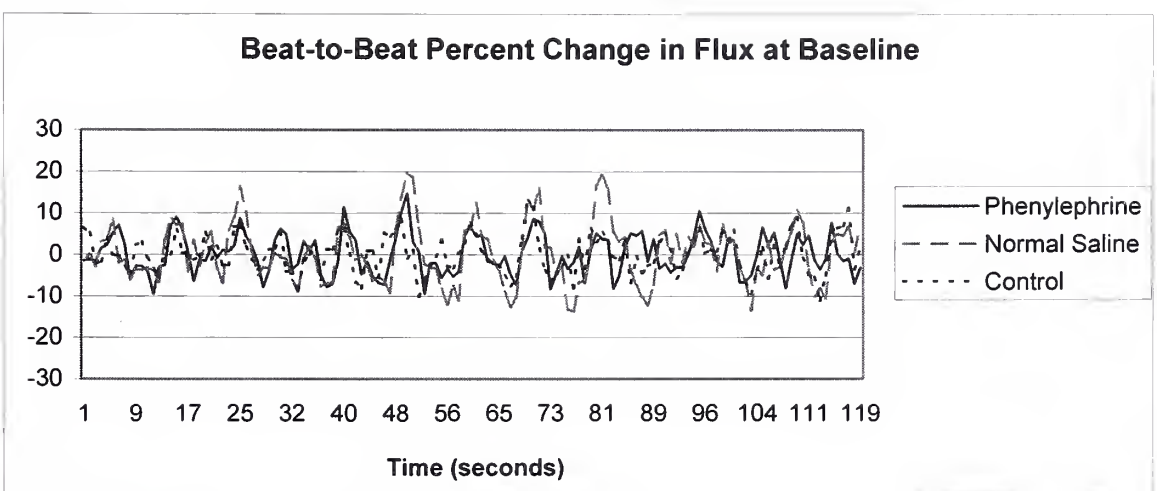
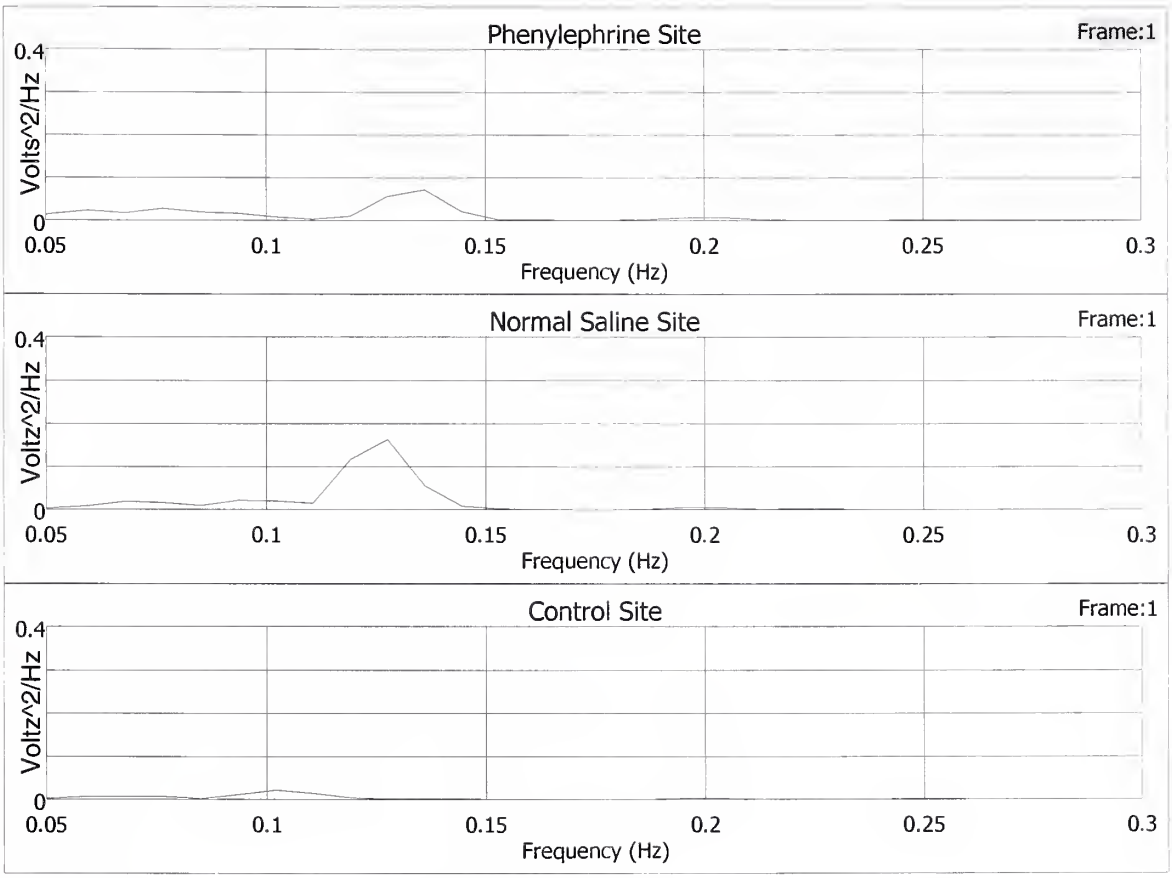


Fig. 3: $APSD_{phenyl}$, $APSD_{NS}$, $APSD_{control}$, and beat-to-beat percent change in flux during **baseline** period.



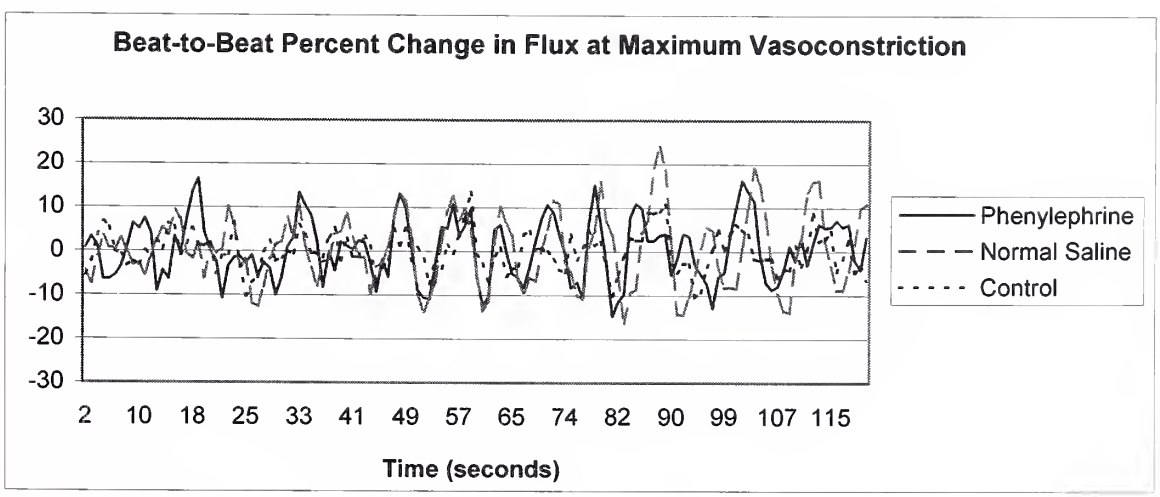
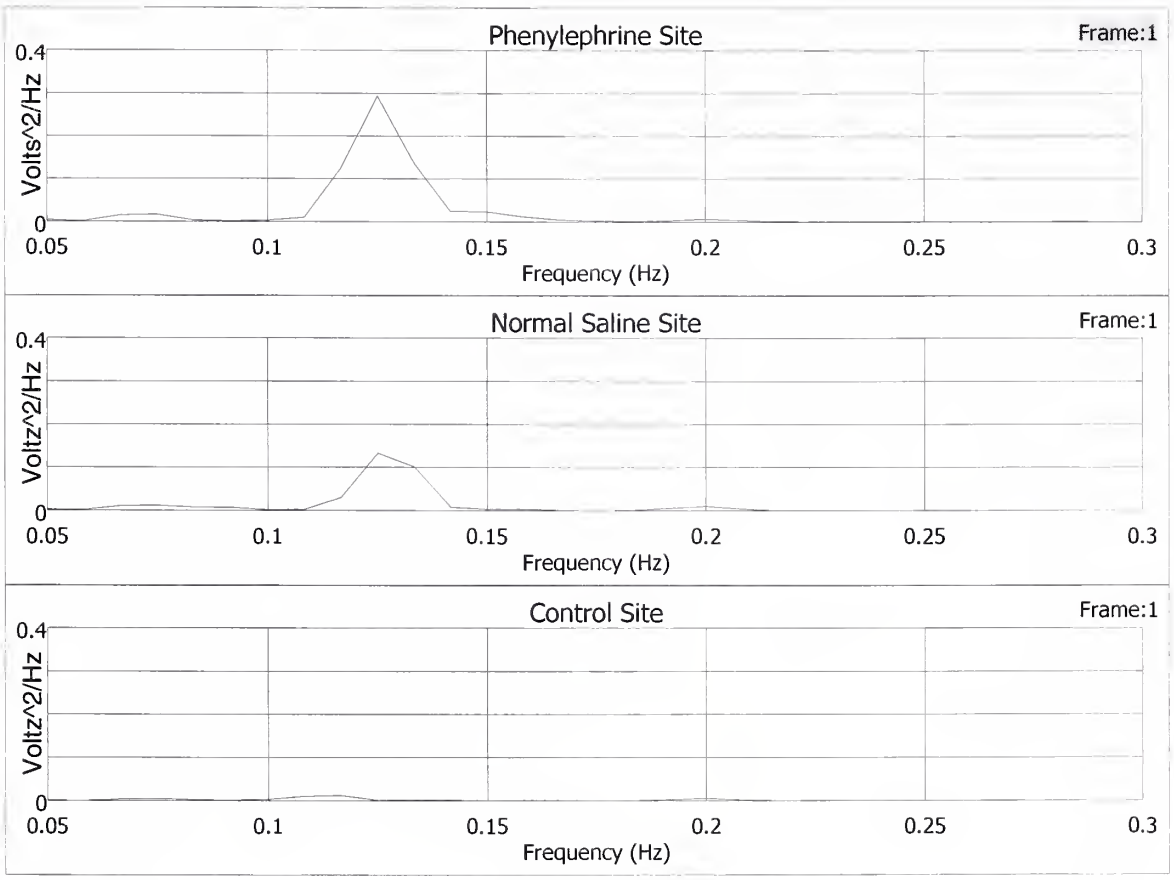


Fig. 4: $APSD_{phenyl}$, $APSD_{NS}$, $APSD_{control}$, and beat-to-beat percent change in flux during **period of maximum vasoconstriction at the phenylephrine site**.



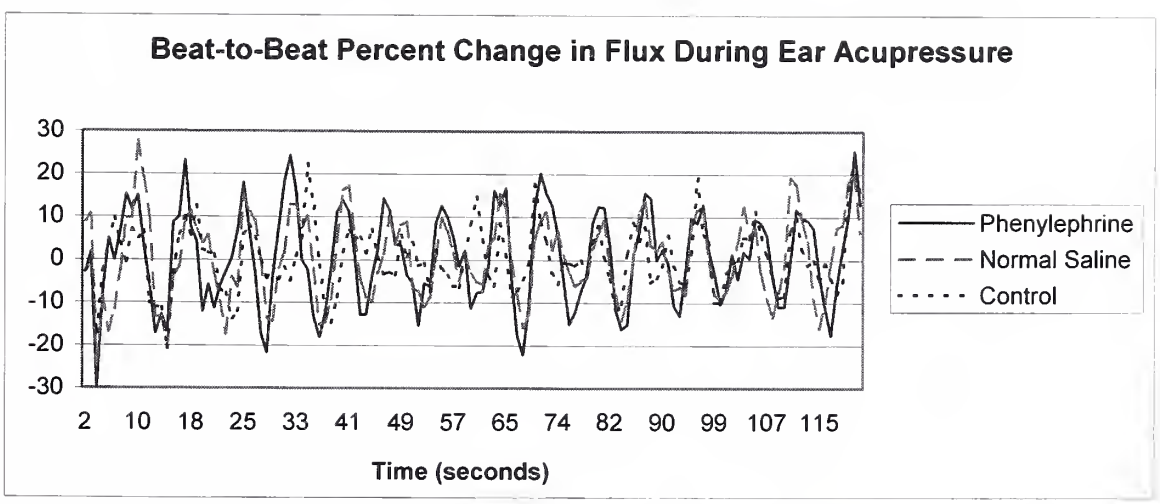
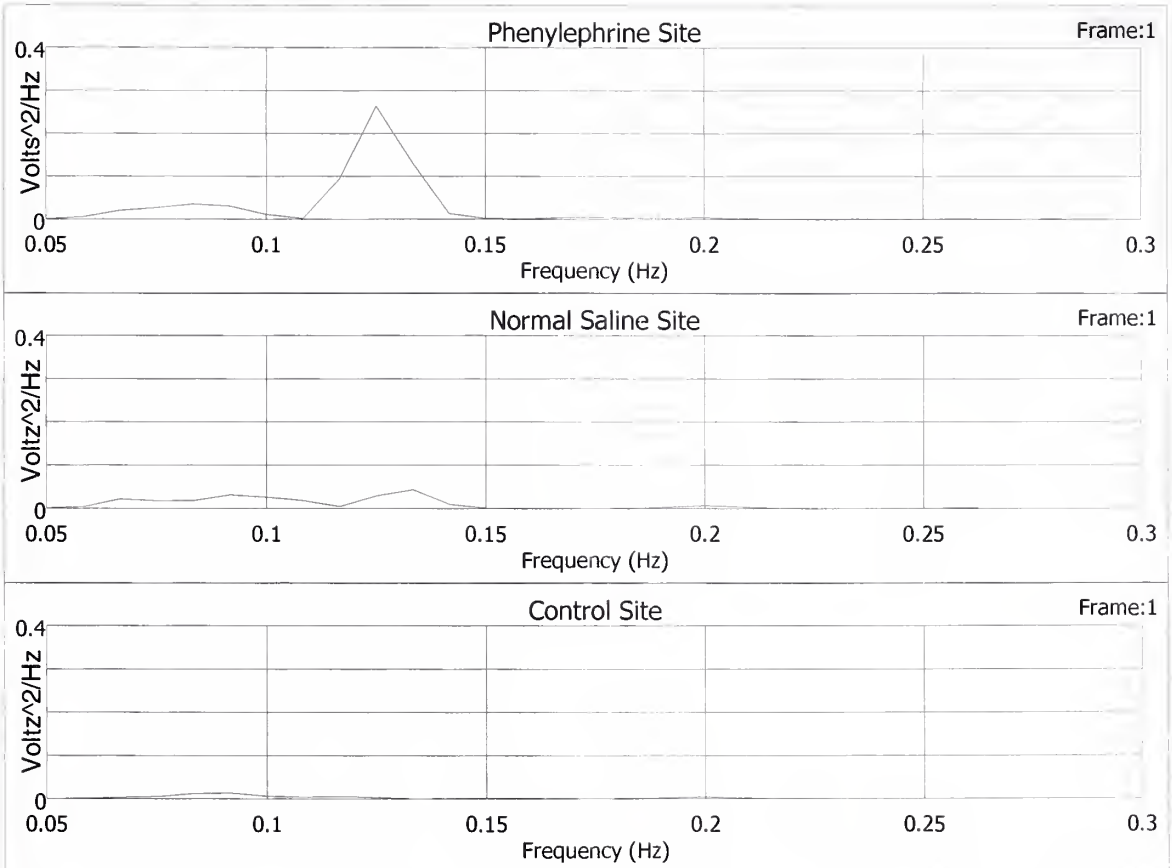


Fig. 5: $APSD_{phenyl}$, $APSD_{NS}$, $APSD_{control}$, and beat-to-beat percent change in flux during **ear acupressure**.



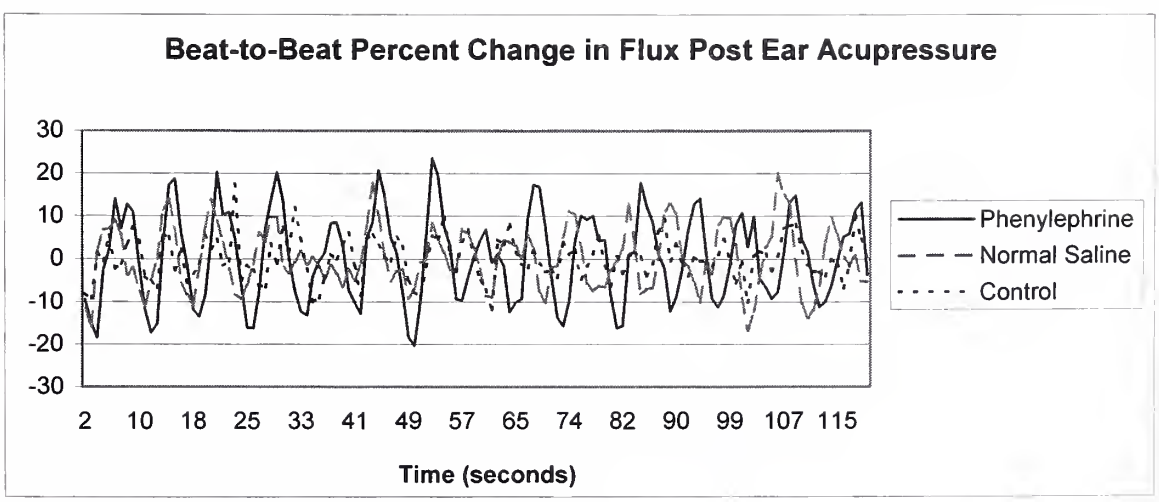


Fig. 6: $APSD_{phenyl}$, $APSD_{NS}$, $APSD_{control}$, and beat-to-beat percent change in flux during **post-acupressure** period.

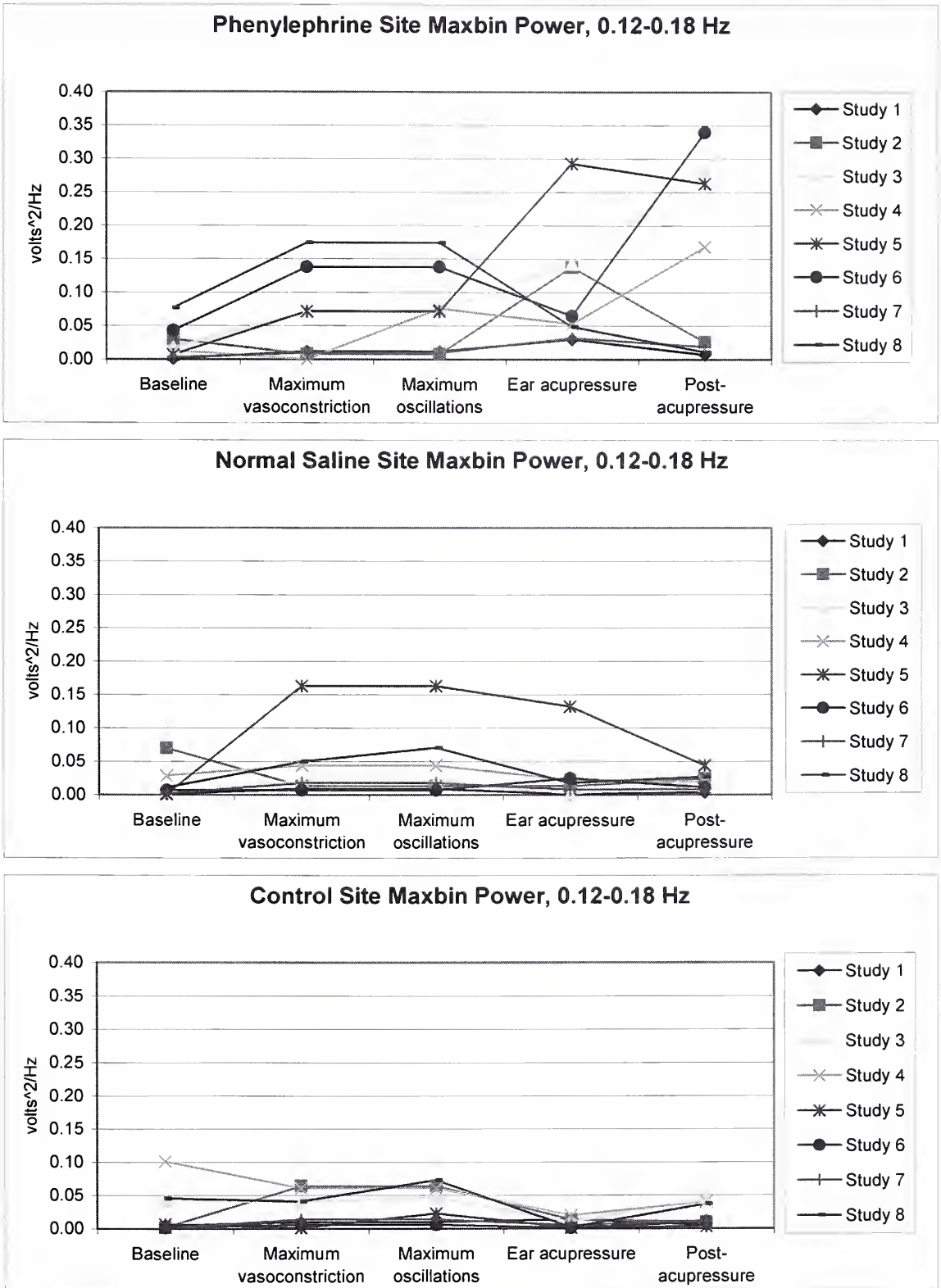


Fig. 7: IF range (0.12-0.18 Hz) maxbin power for all studies at phenylephrine, normal saline, and control sites.

Table 2: IF (0.12-0.18 Hz) maxbin power: mean (standard error of mean) for all studies

	Baseline	Maximum vasoconstriction	Maximum oscillations	Ear acupressure	Post-acupressure
Phenylephrine	0.024 (0.009)	0.061 (0.023)	0.070 (0.022)	0.100 (0.032)	0.140 (0.050)
Normal saline	0.016 (0.008)	0.041 (0.019)	0.043 (0.019)	0.028 (0.015)	0.019 (0.005)
p	0.5	0.5	0.3	0.010*	0.043
p (ln trans)	0.1	0.9	0.3	0.005*	0.033
Control site	0.026 (0.013)	0.030 (0.009)	0.037 (0.010)	0.008 (0.003)	0.020 (0.006)
p	0.9	0.3	0.2	0.025*	0.046
p (ln trans)	0.6	0.8	0.4	0.002*	0.056

Data values indicate the mean maxbin power for all eight studies at the given forehead site and time period. *p* values are for two-tail paired t-test comparing normal saline site vs. phenylephrine site and control site vs. phenylephrine site. The first *p* value in each set represents comparison of the actual maxbin powers between the two sites; the second *p* value represents comparison after *ln* transformation of the maxbin powers for each site in order to minimize the influence of skewness. *p* values retaining significance after Bonferroni's adjustment are indicated with an asterisk.

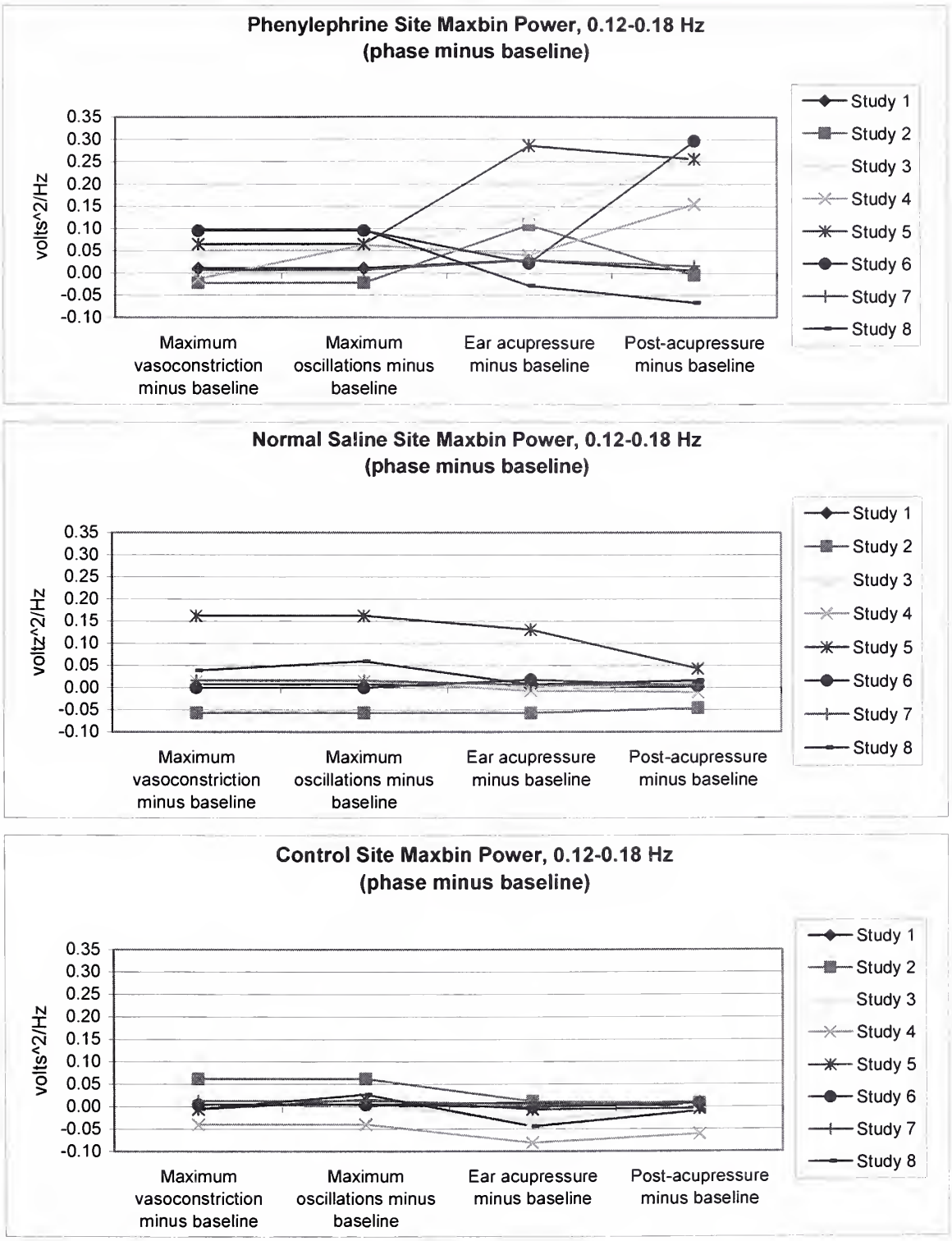


Fig. 8: IF maxbin power, phase-minus-baseline. Data points represent IF maxbin power at given phase minus IF maxbin power at baseline.

Table 3: IF (0.12-0.18 Hz) maxbin power, phase minus baseline: mean (standard error of mean) for all studies

	Maximum vasoconstriction	Maximum oscillations	Ear acupressure	Post-acupressure
Phenylephrine	0.037 (0.017)	0.046 (0.015)	0.076 (0.035)	0.116 (0.051)
Norm saline	0.025 (0.022)	0.028 (0.022)	0.012 (0.019)	0.003 (0.009)
p	0.6	0.4	0.045	0.053
Control site	0.004 (0.010)	0.012 (0.010)	-0.017 (0.011)	-0.005 (0.008)
p	0.2	0.2	0.029	0.053

Data values indicate the mean of (maxbin power at indicated phase minus maxbin power at baseline) for all eight studies. *p* values are for two-tail paired t-test comparing normal saline site vs. phenylephrine site and control site vs. phenylephrine site. No *p* value retained significance after Bonferroni's adjustment.

Table 4: Paired t-test, IF maxbin power at each phase versus baseline

	Maximum vasoconstriction	Maximum oscillations	Ear acupressure	Post-acupressure
Phenylephrine				
p	0.063	0.019	0.063	0.058
p (ln trans)	0.4	0.022	0.008*	0.040
Norm saline				
p	0.3	0.3	0.5	0.7
p (ln trans)	0.08	0.08	0.4	0.1
Control site				
p	0.7	0.3	0.2	0.5
p (ln trans)	0.3	0.043	0.4	0.3

p values are for two-tail paired t-test comparing the IF maxbin power during each time period versus the IF maxbin power at baseline. The first *p* value in each set represents comparison of the actual maxbin powers between the given phase and baseline; the second *p* value represents comparison after *ln* transformation of the maxbin powers at each phase in order to minimize the influence of skewness. *p* values retaining significance after Bonferroni's adjustment are indicated with an asterisk.

Figure 9 plots the mean R-R interval over four time phases for four studies. The "maximum oscillations at each site" phase is omitted from this plot because this phase was different for each of the three forehead sites and therefore did not correlate with one specific ECG time segment. Also shown in this figure is the standard deviation of the R-R interval in the same four studies. Based on the R-R interval plot, there was no consistent change in R-R interval during the course of each study. In particular, there is no obvious increase in R-R interval during the ear acupressure phase, suggesting that there was no systemic vagal effect from acupressure. There was also no consistent change in the variability of the R-R interval, as shown by the plot of standard deviation.

Figure 10 displays the IF range maxbin power of the heart rate for four studies. The "maximum oscillations at each site" phase is omitted from this plot for the same reason as in Figure 9. Only one subject showed an obvious change in IF range maxbin heart rate power, which occurred during the acupressure phase. This subject did not evidence an unusually high IF power at the phenylephrine site, arguing against transmission of these oscillations from the heart.

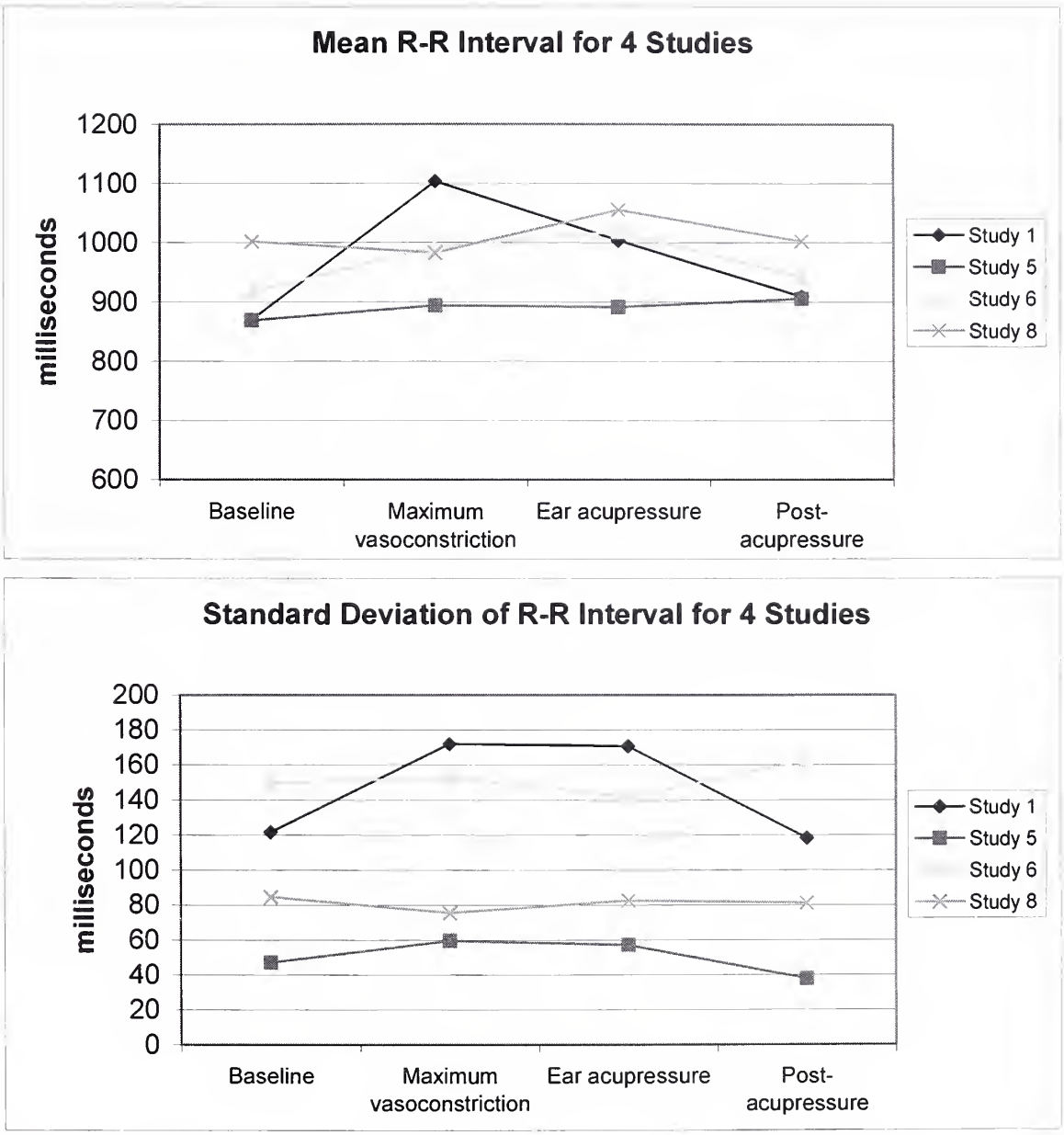


Fig. 9: Mean R-R interval and standard deviation of R-R interval for four studies.

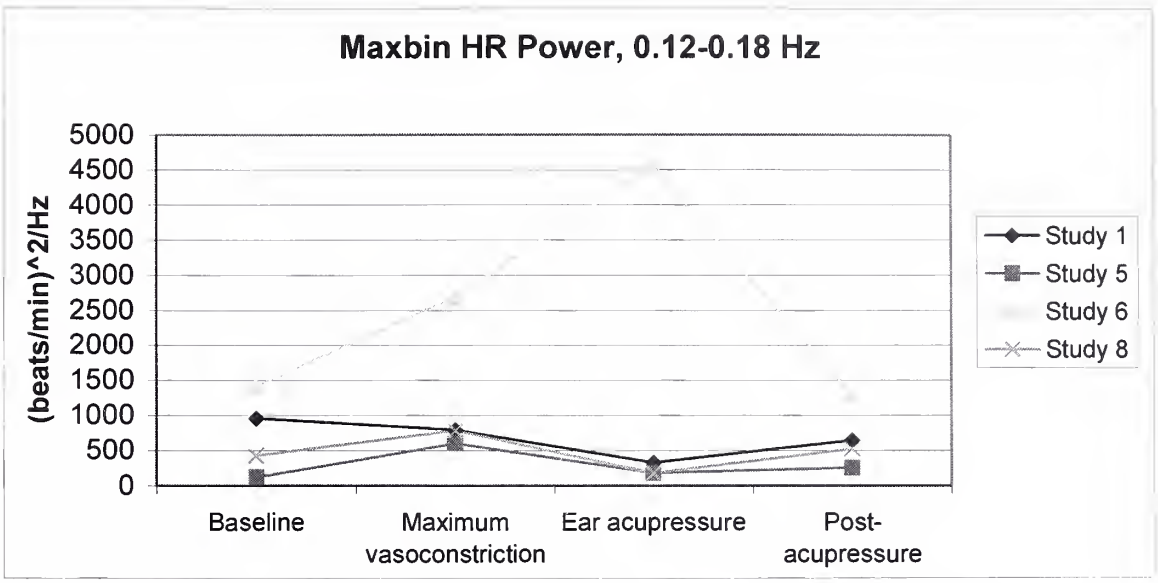


Fig. 10: IF maxbin heart rate power for four studies.

Discussion

The results of this investigation include several interesting findings regarding microvascular cholinergic oscillatory control. We have shown that iontophoretic administration of phenylephrine, when accompanied by an acupressure method known to increase vagal tone, precipitated an increase in the organization and amplitude of oscillatory activity in the forehead microvasculature at a frequency believed to be incompatible with a sympathetic-mediated oscillatory process (i.e., greater than 0.12 Hz). This frequency was also shown to be different from the metronome-controlled respiratory frequency and from heart rate variability. Furthermore, in this study these oscillations appeared in the setting of an average decrease in local microvascular flux of 25% due to the phenylephrine. The present study offers at least three major contributions to current investigations of microvascular cholinergic oscillatory control.

First, this study adds to the understanding of the physiology of microvascular oscillatory activity. Prior studies by Silverman and colleagues demonstrated that systemic administration of phenylephrine elicited forehead microvascular oscillations in the IF range (0.12-0.18 Hz) and that these oscillations were distinct from the respiratory frequency and from heart rate variability (35,42). However, although these studies separated the microvascular oscillations from known systemic forms of periodicity, they did not address whether these oscillations could still be mediated in part by baroreceptor activity, nor did they attempt to localize the components of the reflex in terms of "afferent" or "efferent" limbs. Although the entire pathway (or pathways) remains to be delineated, the current study provides evidence against a baroreceptor-mediated mechanism.

There appear to be two basic components of the microvascular reflex seen in this study. The first component is the vasoactive challenge (phenylephrine), as evidenced by the IF range oscillations being significantly more powerful at the phenylephrine site than at the NS or control sites during the ear acupressure phase. The second component is the acupressure, which we

believe, in agreement with other investigators' findings, led to an increase in vagal output (65). With the introduction of ear acupressure, the phenylephrine and NS sites, which had previously behaved similarly in response to their respective iontophoretic stimuli, suddenly showed significant differences in their IF range maxbin oscillatory powers. Thus, we have shown that IF range microvascular oscillations can be elicited at a local skin site by administering a vasoconstrictive challenge specifically at that site and enhancing vagal output with acupressure. Although this finding is a significant step forward from the prior investigation using systemic phenylephrine, we cannot yet conclude that the entire oscillatory reflex is mediated locally. We have demonstrated that the iontophoresed phenylephrine produced no systemic effects, as evidenced by there being no change in flux at the control site and no consistent change in the R-R interval or in R-R variability. However, given that acupressure was required to elicit the oscillatory activity fully, we cannot yet rule out a central component to the pathway.

As its second major contribution, this study serves as a model which enhances our current understanding of the mechanism of acupuncture. In this study acupressure (a form of acupuncture) was required to elicit fully the local IF microvascular oscillatory effect. While this finding initially came as a surprise to our team, it is consistent with the current knowledge of how acupuncture works. Acupuncture is a much-studied but incompletely understood technique which has been used for over 4,000 years to treat pain as well as a wide variety of medical diseases ranging from asthma to gastrointestinal disorders to mood disorders (70,71). Based on the traditional Chinese principle of opposites and the dynamic balance of the two opposing forces, Yin and Yang, acupuncture has long been considered a technique which works by restoring balance to a biological system whose homeostasis has been disturbed by a disease process (70,72). A complete discussion of the many current biological theories of how acupuncture may exert its effects is beyond the scope of this paper. However, common to many of the theories is that acupuncture has little or no effect on normal function; rather, "only in dysfunction does the 'balancing mechanism' produce clear effects" (72). In light of this

understanding, it seems reasonable to view the three laser Doppler forehead sites in terms of any imbalances in blood flow which may have been imposed on them during the study. The control site received no direct stimulation and, as expected, showed no significant change in its IF range maxbin power throughout the study. The NS site underwent iontophoresis, which led to a variable mild increase in flux in some subjects but did not result in a significant change in IF maxbin power. The phenylephrine site, however, showed evidence of vasoconstriction based on an average 25% decrease in flux following iontophoresis. It was at this site, where blood flow homeostasis was most greatly disturbed, that the IF maxbin power increased significantly during acupressure. In other words, we propose that the phenylephrine forehead site alone was primed, by a vasoconstrictive challenge, to respond to the acupressure-induced increase in vagal tone which mediated the homeostatic oscillatory response. Although the exact chemical nature of this "priming" is unknown, one possibility is that the vasoconstriction leads to an increased local concentration of acetylcholine, which is then present to exert its cholinergic homeostatic effect with the increase in vagal tone.

This analysis suggests that in our study, phenylephrine-induced vasoconstriction created the type of blood flow imbalance which microvascular oscillations may exist to offset. However, several differences between these results and those seen in an earlier study involving systemic administration of phenylephrine call for an explanation. The IF range forehead microvascular oscillations Silverman and colleagues observed upon intravenous infusion of phenylephrine were neither accompanied by nor preceded by any change in forehead flux (35). Additionally, the oscillations were seen without any additional stimuli such as acupressure. These two major differences between the studies, if considered together, may help to explain each other. Although local tissue concentrations of phenylephrine were not measured in either study, the 25% decrease in forehead flux in the present study suggests a higher local drug concentration at the forehead phenylephrine site than may have existed when phenylephrine was administered systemically with no change in forehead flux. If so, it is quite possible that the vasoconstriction induced by the

iontophoresed phenylephrine was greater than the physiological imbalances that microvascular oscillations may exist to offset. The compensatory system may initially have been overwhelmed by the local phenylephrine concentration, and not until parasympathetic tone was increased by ear acupressure was the homeostatic response able to exert a measurable effect. By contrast, with systemic infusion of phenylephrine, the local forehead drug concentration may have created a vasoactive challenge more similar to normal physiological imbalances, i.e., just enough to activate the homeostatic oscillatory response, which in turn prevented a significant decrease in local flow.

Another difference between the two studies lies in the route taken by phenylephrine to reach the microvasculature. When infused intravenously, the phenylephrine began at the lumenal aspect of the microvasculature and from there traveled to its effector site(s). However, the iontophoresed phenylephrine passed through cutaneous and subcutaneous tissue, reaching the outer component of the vessels first. Without a full knowledge of the receptors and effector sites involved in the oscillatory pathway, we cannot yet describe how the system is affected by the route of drug travel. Nevertheless, these differences between methods of administration should be kept in mind during future studies, as they may help to explain any differences in the oscillatory effect, particularly in the time course of the response.

The third major potential contribution of this study is toward the development of a method of monitoring and testing cholinergic oscillatory control of the microvasculature in the clinical setting. The interest in such a technique stems from multiple investigations which have shown that autonomic dysfunction is responsible for considerable morbidity and mortality among patients with cardiovascular diseases such as diabetes and hypertension (66,68). There has been recent interest in the monitoring of microcirculatory oscillations as a method of detecting autonomic dysfunction in patients before it becomes clinically apparent (50,51). In fact, several investigators have not only demonstrated impairment of sympathetic-frequency microvascular oscillations in diabetic patients, but also have shown that the degree of impairment correlates with

the duration of diabetes, the resting heart rate variability, and the median motor and sensory nerve conduction velocities (50,51,52). Adding to these tests of sympathetic dysfunction, the ability to detect the presence or absence of a parasympathetic microvascular homeostatic response to vasoconstriction would enable the testing of both divisions of the autonomic nervous system and thereby increase the flexibility and sensitivity of such testing. The next critical step in the development of this form of testing is to assess whether patients with advanced diabetes show evidence of IF range microvascular oscillations in response to a vasoconstrictive challenge.

One major limitation of the present study is that the laser Doppler probe shows variability in its absolute flux measurements at different forehead skin sites in the same subject. These variations are presumably due to site-dependent differences in the microvascular anatomy beneath the probe (69). We have attempted to overcome this problem in several ways. When determining the significance of changes in flux measured by each probe, we compared the flux during each phase with the flux at baseline so that changes would be detected at each site regardless of differences between probes in the measurement of absolute flux. In addition, we examined the beat-to-beat percent change in flux for each phase of interest rather than considering only absolute flux. Finally, when evaluating maxbin oscillatory power (which is affected by absolute flux), we examined changes in power over time and, for each forehead site, compared the maxbin power at each phase with the power at baseline.

A second limitation of this study is that we have not yet confirmed the cholinergic nature of the microvascular oscillations by administering atropine (as had been done with intravenous phenylephrine). However, having now demonstrated the development of IF range microvascular oscillations within minutes after iontophoresis, followed by their enhancement with acupressure ten minutes after iontophoresis, we feel better prepared to administer atropine having some knowledge of the time course of the oscillatory effect. Additionally, the observed oscillations show a frequency that is outside the range within which the sympathetic nervous system is believed to be capable of mediating, providing indirect evidence of their parasympathetic nature.

Thus the present study has contributed to the understanding of the physiology of microvascular oscillatory activity, to the theories regarding the mechanism of acupuncture, and to the potential development of a clinical test for autonomic dysfunction. Further studies will include the administration of atropine, which we hope will provide further evidence of a parasympathetic mediation of the IF microvascular oscillations. Additionally, studies involving patients with known advanced diabetes will reveal whether such patients have lost this compensatory mechanism. The utility of a clinical test for both sympathetic and parasympathetic dysfunction, as well as the potential for characterizing a previously unrecognized homeostatic pathway, will motivate further research of this fascinating process.

References

1. Anrep, G.V., Pascual, W., and Rössler, R. 1936. Respiratory variations of the heart rate. I. The reflex mechanism of the respiratory arrhythmia. *Proc R Soc Lond B Biol Sci.* 119:191-217.

2. Schweitzer, A. 1945. Rhythmical fluctuations of the arterial blood pressure. *J Physiol (Lond).* 104:25P.

3. Hyndman, B.W., Kitney, R.I., and Sayers, B.McA. 1971. Spontaneous rhythms in physiological control systems. *Nature.* 233:339-341.

4. Sayers, B.McA. 1973. Analysis of heart rate variability. *Ergonomics.* 16:17-32.

5. Pomeranz, B., Macaulay, R.J.B., Caudill, M.A., Kutz, I., Adam, D. *et al.* 1985. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol.* 248:H151-53.

6. Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., *et al.* 1986. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res.* 59:178-193.

7. Rimoldi, O., Pierini, S., Ferrari, A., Cerutti, S., Pagani, M., *et al.* 1990. Analysis of short-term oscillations of R-R and arterial pressure in conscious dogs. *Am J Physiol.* 258: H967-76.

8. Rosenbaum, M., and Race, D. 1968. Frequency-response characteristics of vascular resistance vessels. *Am J Physiol.* 215:1397-1402.

9. Guyton, A.C. and Hall, J.E. 1996. Overview of the circulation, medical physics of pressure, flow and resistance. In *Textbook of Medical Physiology, Ninth Edition.* A.C. Guyton, and J.E. Hall, editors. Philadelphia: W.B. Saunders Co. 161-169.

10. Bernardi, L., Hayoz, D., Wenzel, R., Passino, C., Calciati, A., *et al.* 1997. Synchronous and baroreceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. *Am J Physiol.* 273:H1867-H1878.

11. Nicoll, P.A., and Webb, R.L. 1946. Blood circulation in the subcutaneous tissue of the living bat's wing. *Ann NY Acad Sci.* 46:697-709.

12. Nicoll, P.A., and Webb, R.L. 1955. Vascular patterns and active vasomotion as determiners of flow through minute vessels. *Angiology.* 6:291-310.

13. Fagrell, B., Intaglietta, M., and Östergren, J. 1980. Relative hematocrit in human skin capillaries and its relation to capillary blood flow velocity. *Microvas Research.* 20(3):327-35.

14. Nilsson, G.E., Tenland, T., and Öberg, P.Å. 1980. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng.* 27:12-19.

15. Nilsson, G.E., Tenland, T., and Öberg, P.Å. 1980. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *IEEE Trans Biomed Eng.* 27:597-604.

16. Tenland, T., Salerud, E.G., Nilsson, G.E., and Öberg, P.Å. 1983. Spatial and temporal variations in human skin blood flow. *Int J Microcirc Clin Exp.* 2:15-24.

17. Colantuoni, A., Bertuglia, S., and Intaglietta, M. 1994. Microvascular vasomotion: origin of laser Doppler flux motion. *Int J Microcirc Clin Exp.* 14:151-158.

18. Wilkin, J.K. 1989. Poiseuille, periodicity, and perfusion: rhythmic oscillatory vasomotion in the skin. *J Invest Dermatol.* 93:113S-118S.

19. Salerud, E.G., Tenland, T., Nilsson, G.E., and Öberg, P.Å. 1983. Rhythmical variations in human skin blood flow. *Int J Microcirc Clin Exp.* 2:91-102.

20. Bernardi, L., Rossi, M., Fratino, P., Finardi, G., Mevio, E. *et al.* 1989. Relationship between phasic changes in human skin blood flow and autonomic tone. *Microvasc Res.* 37:16-27.

21. Lossius, K. and Eriksen, M. 1995. Spontaneous flow waves detected by laser Doppler in human skin. *Microvasc Res.* 50:94-104.

22. Intaglietta, M. 1981. Vasomotor activity, time-dependent fluid exchange and tissue pressure. *Microvasc Res.* 21:153-164.

23. Intaglietta, M. and Gross, J.F. 1982. Vasomotion, tissue fluid flow and the formation of lymph. *Int J Microcirc Clin Exp.* 1:55-65.

24. Johnsson, P.C., and Wayland, H. 1967. Regulation of blood flow in single capillaries. *Am J Physiol.* 212(6):1405-1415.

25. Siegel, G. 1983. Principles of vascular rhythmogenesis. *Prog Appl Microcirc.* 3:40-62.

26. Mevio, E., and Bernardi, L. 1994. Phasic changes in human nasal and skin blood flow: relationship with autonomic tone. *Ann Otol Rhinol Laryngol.* 103:789-795.

27. Silverman, D.G., Jotkowitz, A.B., Freemer, M., Gutter, V., O'Connor, T., *et al.* 1994. Peripheral assessment of phenylephrine-induced vasoconstriction by laser Doppler flowmetry and its potential relevance to homeostatic mechanisms. *Circulation.* 90:23-26.

28. Lindblad, L.E., and Ekenvall, L. 1986. Alpha-adrenoceptors in the vessles of human finger skin. *Acta Physiol Scand.* 128:219-222.

29. Edfeldt, H., and Lundvall, J. 1993. Sympathetic baroreflex control of vascular resistance in comfortably warm man: analyses of neurogenic constrictor responses in the resting forearm and in its separate skeletal muscle and skin tissue compartments. *Acta Physiol Scand.* 147:437-447.

30. Johnson, J.M. 1989. Circulation to the skin. In *Textbook of Physiology, 21st Edition.* H.D. Patton, A.F. Fuchs, B. Hille, A.M. Scher, R. Steiner, editors. Philadelphia: W.B. Saunders. 898-910.

31. Kellogg, D.L. Jr., Johnson, J.M., and Kosiba, W.A. 19909. Baroreflex control of the cutaneous active vasodilator system in humans. *Circ Res.* 66:1420-1426.

32. Beiser, G.D., Zelis, R., Epstein, S.E., Mason, D.T., and Braunwald, E. 1970. The role of skin and muscle resistance vessels in reflexes mediated by the baroreceptor system. *J Clin Invest.* 49:225-231.

33. Mengesha, Y.A., and Bell, G.H. 1979. Forearm and finger blood flow responses to passive body tilts. *J Appl Physiol.* 46:288-292.

34. Levine, M.A.H., Leenan, F.H.H. 1992. Role of vagal activity in the cardiovascular responses to phenylephrine in man. *Br J Clin Pharmacol.* 33:333-336.

35. Silverman, D.G., Stout, R.G., Lee, F.A., and Ferneini, E.M. 2001. Detection and characterization of cholinergic oscillatory control in the forehead microvasculature in response to systemic α-agonist infusion in healthy volunteers. *Microvasc Res.* 61:144-147.

36. Bell, C. 1996. Cholinergic vasodilator mechanisms. In *Nervous Control of Blood Vessels.* T. bennett and S.M. Gardiner, editors. Harwood Academic, Australia. 59-74.

37. Guyton, A.C., and Hall, J.E. 1996. *Textbook of Medical Physiology.* A.C. Guyton and J.E. Hall, editors. Philadelphia: W.B. Saunders Co. 209-220.

38. Florence, V. and Bevan, J.A. 1979. Biochemical determinations of cholinergic innevation in cerebral arteries. *Circ Res.* 45:212-217.

39. Moore, S.J., and Shore, A.J. 1996. Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: possible mechanisms. *J Physiol.* 496 Pt. 2:521-542.

40. Imaizumi, T., Takeshita, A., Suzuki, S., Yoshida, M., Ando, S., *et al.* 1990. Age-independent forearm vasodilatation by acetylcholine and adenosine 5'-triphosphate in humans. *Clin Sci.* 78:89-93.

41. Izumi, H. 1994. Reflex parasympathetic vasodilatation in facial skin. *Gen Pharmac.* 26:237-244.

42. Silverman, D.G., and Stout, R.G. 2002. Distinction between atropine-sensitive control of microvascular and cardiac oscillatory activity. *Microvasc Res.* In press.

43. Hutcheson, I.R., and Griffith, T.M. 1991. Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *Am J Physiol.* 261:H257-H262.

44. Rubanyi, G.M. 1988. Endothelium-dependent pressure-induced contraction of isolated canine carotid arteries. *Am J Physiol.* 255:H783-H788.

45. Griffith, T.M., Edwards, D.H., Davies, R.L., Harrison, T.J., and Evans, K.T. 1987. EDRF coordinates the behaviour of vascular resistance vessels. *Nature.* 329:442-445.

46. Keys, A., and Violante, A. 1941. The cardio-circulatory effects in man of neo-synephrin. *Clin Invest.* 20:1-12.

47. Cocks, T.M., and Angus, J.A. 1983. Endothelial-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature.* 305:627-630.

48. Furchgott, R.F., and Zawadzki, J.V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 28:373-376.

49. Radaelli, A., Bernardi, L., Valle, F., Leuzzi, S., Salvucci, F., *et al.* 1994. Cardiovascular autonomic modulation in essential hypertension: effect of tilting. *Hypertension.* 24:556-563.

50. Stansberry, K.B., Shapiro, S.A., Hill, M.A., McNitt, P.M., Meyer, M.D., *et al.* 1996. Impaired peripheral vasomotion in diabetes. *Diabetes Care.* 19:715-721.

51. Bernardi, L., Rossi, M., Leuzzi, S., Mevio, E., Fornasari, G., *et al.* 1997. Reduction of 0.1 Hz microcirculatory fluctuations as evidence of sympathetic dysfunction in insulin-dependent diabetes. *Cardiovasc Res.* 34:185-191.

52. Aso, Y., Inukai, T., and Takemura, Y. 1997. Evaluation of skin vasomotor reflexes in response to deep inspiration in diabetic patients by laser Doppler flowmetry. *Diabetes Care.* 20:1324-1328.

53. Bernardi, L., Calciati, A., Marti, G., Ricordi, L., Rossi, M., *et al.* 1986. Bedside diagnosis of cardiac autonomic damage by computerized analysis of heart rate-respiration relationship. *Acta Diabetol Lat.* 23:141-154.

54. van den Akker, T.J., Koeleman, A.S., Hogenhuis, L.A., and Rompelman, O. 1983. Heart rate variability and blood pressure oscillations in diabetics with autonomic neuropathy. *Automedica.* 4:201-208.

55. Morris, S.J., Shore, A.C., and Tooke, J.E. 1995. Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. *Diabetologia.* 38:1337-1344.

56. Watkins, P.J., and Edmonds, M.E. 1983. Sympathetic nerve failure in diabetes. *Diabetologia.* 25:73-77.

57. Sloan, J.B., and Soltani, K. 1986. Iontophoresis in dermatology. *J Am Acad Dermatol.* 15:671-684.

58. Kassan, D.G., Lynch, A.M., and Stiller, M.J. 1996. Physical enhancement of dermatologic drug delivery: Iontophoresis and phonophoresis. *J Am Acad Dermatol.* 34:657-666.

59. Costello, C.T., and Jeske, A.H. 1995. Iontophoresis: Applications in transdermal medication delivery. *Phys Ther.* 75:554-563.

60. Kellogg, D.L. Jr., Pérgola, P.E., Piest, K.L., Kosiba, W.A., Crandall, C.G., *et al.* 1995. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res.* 77:1222-1228.

61. Grossmann, M., Jamieson, M.J., Kellogg, D.L. Jr., Kosiba, W.A., Pergola, P.E., *et al.* 1995. The effect of iontophoresis on the cutaneous vasculature: Evidence for current-induced hyperemia. *Microvasc Res.* 50:444-452.

62. Noon, J.P., Walker, B.R., Hand, M.F., and Webb, D.J. 1998. Studies with iontophoretic administration of drugs to human dermal vessels *in vivo*: cholinergic vasodilatation is mediated by dilator prostanoids rather than nitric oxide. *Br J Clin Pharmacol.* 45:545-550.

63. Ekenvall, L., and Lindblad, L.E. 1986. Is vibration white finger a primary sympathetic nerve injury? *Br J Ind Med.* 43:702-706.

64. Kellogg, D.L. Jr, Johnson, J.M., and Kosiba, W.A. 1989. Selective abolition of adrenergic vasoconstrictor responses in skin by local iontophoresis of bretylium. *Heart Circ Physiol.* 26:H1599-H1606.

65. Haker, E., Egekvist, H., and Bjerring, P. 2000. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. *J Autonomic Nerv Syst.* 79:52-59.

66. Stout, R.G., Fontes, M.L., and Silverman, D.G. 1996. The evaluation of sympathetic and parasympathetic activity by spectral analysis. *Anesthetic Pharmacology and Physiology Review.* 4:96-110.

67. Rice, J.A. 1995. Experimental Design. In *Mathematical Statistics and Data Analysis, 2nd ed.* Belmont: Wadsworth, Inc. 423-424.

68. O'Brien, I.A., McFadden, J.P., and Corrall, R.J. 1991. The influence of autonomic diabetic neuropathy on mortality in insulin-dependent diabetes. *Q J Med.* 79:495-502.

69. Braverman, I.M., Keh, A., and Goldminz, D. 1990. Correlation of laser Doppler wave patterns with underlying microvascular anatomy. *J Invest Derm.* 95:283-286.

70. Raj, P.P. 1986. Acupuncture. In *Practical Management of Pain.* Chicago: Year Book Medical Publishers, Inc. 799-813.

71. Spielman, F.J. 2001. Acupuncture: What's the point? *Am J Anesthesiology.* 28:306-308.

72. Andersson, S., and Lundeberg, T. 1995. Acupuncture—from empiricism to science: Functional background to acupuncture effects in pain and disease. *Medical Hypotheses.* 45:271-281.

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